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DETERMINATION OF THE SUSCEPTIBILITY OF *AEROMONAS SALMONICIDA* TO SULFONAMIDES AND ANTIBIOTICS, WITH A SUMMARY REPORT ON THE TREATMENT AND PREVENTION OF FURUNCULOSIS

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IT IS A WELL-ESTABLISHED FACT THAT pathogenic bacteria are capable of producing mutants that have an increased resistance to drugs used for their control. Therefore, it is only natural that sulfonamide-resistant variants of Aeromonas salmonicida[1/] have appeared since 1946, when sulfonamide therapy of furunculosis was introduced by Gutsell.

During the early years of sulfonamide treatment, results were almost universally excellent. More recently, however, the Leetown laboratory has received frequent reports of outbreaks which failed to respond satisfactorily. An outbreak at Leetown in 1955 provided the initial material for the study of the problem of drug resistance. Experiments made for the selection of a method which would permit rapid and reliable determination of drug-resistant variants of A. salmonicida have been described (Snieszko and Bullock, in press). The performance of such tests, as well as revised recommendations for the control of furunculosis in a trout or salmon hatchery, is described here.

There are two main reasons why bacteria may be resistant to sulfonamides or other drugs in vitro (in test tube, outside the infected host): (1) A hereditary resistance may have been acquired from exposure of bacteria to the drug; (2) the culture medium on which the organisms are grown may contain a substance which blocks the action of the drug. Sulfonamides and some of the antibiotics do not kill bacteria but hinder the growth and reproduction (bacteriostasis) and in this way help the host to overcome the infection. The composition of the medium is extremely important to the detection of resistance to sulfonamides, and the technique of the test must be rigidly standardized in order to furnish reliable results. Media used in such tests must support good growth of the bacteria involved and, at the same time, should be relatively free from substances which are directly antagonistic to the action of sulfonamides or from which antagonists can be synthesized by the tested organism.

There are two generally accepted methods for determining the sensitivity of an organism to sulfonamides or antibiotics. In one method, a serial dilution of the drug is prepared in a suitable liquid medium. Test tubes are inoculated with a standardized suspension of bacteria and, after proper incubation, the growth of bacteria is determined visually or photometrically. The second method makes use of an agar medium. This is distributed in petri plates and inoculated in a

[1/] The new generic name Aeromonas, instead of Bacterium, is used in accordance with current requirements of bacterial taxonomy.

standardized way, and glass or metal rings are deposited on its surface (the Oxford cup method). Exact quantities of known dilutions of the drug are placed within the glass rings. After the incubation period, the presence and, to some degree, the width of a zone free from bacterial growth indicate the effectiveness of the drug or the sensitivity of the bacterium tested. Recently the Oxford cup method has been simplified by replacing the rings with standard filter-paper discs impregnated with known quantities of drugs. Discs can be obtained commercially with nodes spaced in a ring or as tips on spokes in a wheel pattern. These multiple discs are particularly suitable for rapid screening of many drugs.

In the Leetown laboratory, the method of serial dilutions gave very good results when the medium was nutrient broth (Bacto) buffered with 0.5 percent of secondary sodium phosphate and adjusted with sodium hydroxide to pH 8.0. Sensitive strains of A. salmonicida were affected by as little as 10 p.p.m. (1 milligram percent). Although it is the more accurate, the procedure employing liquid media and carefully standardized solutions of sulfonamides is too cumbersome for routine laboratory or field use. Therefore, experiments were made to determine conditions permitting the use of agar media and commercially available paper discs containing various drugs. It was found that the standard medium used to diagnose furunculosis at this station (see Appendix) did not permit differentiation of the sulfa-sensitive and sulfa-resistant strains of A. salmonicida unless buffered with sodium phosphate and adjusted to pH 8.0.

Two of the commercially available agar media, the trypticase soy agar and the Mueller Hinton medium, have proved very satisfactory. Trypticase soy agar is preferred because A. salmonicida grows well and produces the characteristic brown pigment. This agar also permits the performance of the paraphenylenediamine test recommended by Griffin (1952). Therefore, trypticase soy agar should be considered as the medium of choice because it is just as good for the diagnosis of furunculosis as the Leetown standard furunculosis medium and is also very suitable for determining the sensitivity to sulfonamides. For the determination of sensitivity to antibiotics, however, the standard furunculosis agar or the Mueller Hinton agar is preferable.

PERFORMANCE OF THE TESTS

Sulfonamides

Tests for the sensitivity to sulfonamides can be performed on petri plates with single or multiple discs, on agar slants with single discs, or in media containing solutions of sulfonamides.

The most reliable results can be obtained by using media distributed in petri plates. The selected medium should be prepared either in screw-capped test tubes (each containing 20 milliliters of the medium) or in screw-capped bottles (each containing 100 milliliters). Media in screw-capped containers can be stored for many months or even for several years if they have not visibly evaporated and are free from contamination. Immediately before use, media should be liquefied in boiling water or steam and distributed into sterilized glass or plastic petri plates, about 20 milliliters of medium per plate (10 centimeters in diameter).

As soon as the medium is well solidified, it should be evenly inoculated with a suspension of the bacteria to be tested. Best results are obtained from vigorous pure cultures. Growth from a solid medium may be used. This should be scraped with a sterile loop and suspended in 1 or 2 milliliters of sterile water to form a visibly opaque suspension. A 24-hour-old culture in a liquid medium can also be used. Trypticase soy, Mueller Hinton, standard furunculosis medium, or even nutrient broth--all, of course, without agar--can be used as liquid media (see Appendix). Inoculation can be made most effectively with a sterile cotton swab on a wooden applicator. As soon as the plate or plates are inoculated, single- or multiple-sensitivity filter-paper discs should be deposited on the surface of the

medium and incubated at 20° to 22° C. (68° to 72° F.) for 24 to 48 hours. If no incubator is available, any clean drawer or box will do. Petri plates should be incubated with bottoms up.

If the results are urgently needed, material taken directly from a fish can be used as inoculum. A sterile cotton swab can be inserted into a furuncle or kidney, or smeared with blood, and used to inoculate the medium. If the test shows that bacteria are resistant to sulfonamides, the results should be considered as tentative, because a mixed culture may be present or traces of blood or tissue may act as antagonists of sulfonamides. A clear zone means that whatever bacteria are present, in pure or mixed culture, are sensitive to sulfonamides in vitro. Regardless of the results obtained in this preliminary test, bacteria should subsequently be isolated and a pure culture be tested.

It has been found that once bacteria--and particularly gram-negative bacteria (for example, A. salmonicida)--become resistant to one sulfonamide, they usually become resistant to other sulfonamides. This makes it permissible to use one disc with one sulfonamide only. The most reliable results have been obtained by using discs of any of the following: sulfadiazine, sulfisoxazole (Gantrisin), thiosulfil, and sulfamerazine. For best results, the quantity of the drug per disc should be adapted to the medium, as follows:

Medium	Micrograms of sulfonamide per disc
Trypticase soy agar----	250 to 500
Mueller Hinton agar----	100 to 250
Standard furunculosis agar medium (buffered and alkalized)-------	500 to 1,000

Where use of petri plates is impractical, the test can be performed with media in the form of agar slants. If standard bacteriological (preferably screw-capped) test tubes (18 by 150 millimeters outside diameter) are used, they should contain about 10 milliliters of medium. Media can either be stored in a slanted form or be stored as stabs and dissolved and slanted just before use. Slants should not be visibly dried out. Inoculation should be made with a loop containing a suspension of bacteria, growth scraped from a solid medium (single colony preferred), or pathological material from a diseased fish. Slants should be inoculated by making one continuous, straight stroke from just above the water of condensation, if any, to the top of the slant. As soon as the medium has been inoculated, a single sensitivity disc should be deposited in the center of the slant with a sterile scalpel, a narrow spatula, forceps, needle, or inoculating loop used as a spatula. Sterilization can be accomplished by immersing in 75 percent (about) alcohol and burning off the excess. Correct deposition of the disc requires some experience and a steady hand. Under no circumstances should the disc touch any part of the slant except the spot intended.

When the test is made in petri plates, a circular zone will appear around the disc if bacteria are drug-sensitive, if the medium has the correct composition, and if the concentration of sulfonamide in the disc is adequate. On agar slants, the clear zone will appear as an area free from bacterial growth just above and below the disc. It is possible that with some media, with discs containing higher quantities of sulfonamides, and with very sensitive cultures, the zones may be so wide that no growth will appear. In order to avoid such complications, a test tube of the same medium should be inoculated but no disc inserted.

The test for sensitivity to sulfonamides can be combined with the paraphenylenediamine test (Griffin 1952). The brown pigment that is characteristic of A. salmonicida usually appears in 2- to 3-day-old cultures grown on the standard furunculosis medium and on trypticase soy agar. On the Mueller Hinton medium, the appearance of brown pigment is either slow or absent. The paraphenylenediamine test is performed by placing a drop of 1 percent aqueous solution of the reagent on the surface of a 24-hour-old growth of

TABLE 1.--Growth of sulfonamide-sensitive and sulfonamide-resistant cultures of Aeromonas salmonicida on media containing sodium sulfadiazine

Medium	Dilution of sodium sulfadiazine					Remarks
	1:500	1:1,000	1:2,000	1:4,000	None (Control)	
			Sulfonamide-sensitive cultures			
Trypticase soy agar--	1/ 0	1/ 0	1/ 0	1/ 0	++++	Brown pigment present in control.
Mueller Hinton agar--	0	0	0	0	++++	Brown pigment absent in control.
Standard furunculosis medium.	+	++	+++	+++	++++	Brown pigment present only in control.
			Sulfonamide-resistant cultures			
Trypticase soy agar--	++++	++++	++++	++++	++++	Brown pigment absent only in 1:500.
Mueller Hinton agar--	++++	++++	++++	++++	++++	Brown pigment absent in all tubes.
Standard furunculosis medium.	++++	++++	++++	++++	++++	Brown pigment absent only in 1:500.

1/ Scanty growth appeared after 3 days of incubation.

A. salmonicida on the standard furunculosis medium or on trypticase soy agar.

Drugs, instead of paper discs containing sulfonamides, can be added to agar media before sterilization. In a recent test, sodium sulfadiazine was added to several media. After sterilization, the media were slanted and the surfaces inoculated with suspensions of either sensitive or resistant strains. The results are presented in table 1.

For practical purposes the test should be performed with trypticase soy agar or buffered and alkalized standard furunculosis medium. Sodium sulfamerazine should be added to the medium at a rate of 1:500 or 1:1,000. Media should be sterilized in the autoclave at 15 pounds for 15 to 20 minutes, slanted, and stored in a refrigerator. The maximum storage time of the medium has not been determined. Media stored for several weeks have been used with good results. When the tube method is used, a control tube (containing no drug) should also be inoculated. If within 24 to 48 hours good growth appears in the control tube but is absent--or nearly so--in the medium with the drug, the culture is sensitive. If good growth appears in both tubes, the culture is resistant to sulfonamides. The dependability of the test can be increased by inoculating additional slants with known drug-susceptible and drug-resistant cultures of A. salmonicida. In test tubes in which growth is not inhibited by the drug, the typical brown pigment should appear within 2 to 3 days.

Antibiotics

Antibiotics have also been recommended for the treatment of furunculosis and other diseases of fish (Snieszko 1954). During 1956, however, there were two occasions when strains of A. salmonicida resistant to oxytetracycline (terramycin)

were isolated from outbreaks of furunculosis treated with this antibiotic. There is no correlation between sensitivity of bacteria to antibiotics and sensitivity of bacteria to sulfonamides, but among pathogenic bacteria there occurs a cross resistance to chemically related antibiotics.

The methods of determining the susceptibility of bacteria to antibiotics are basically the same as those used with sulfonamides. It is generally accepted that the selection of proper media is not so important for determining sensitivity of bacteria to antibiotics, and also that the results which are obtained from the test in vitro are more reliable as guides for treatment.

In vitro tests made with a large number of sulfonamide-sensitive and sulfonamide-resistant strains of A. salmonicida have shown no relationship between the sensitivities to these two groups of drugs.

Sensitivity tests run in standard furunculosis agar (not buffered or alkalized) and with star-shaped multiple-sensitivity discs gave the results shown in table 2.

It can be seen (table 2) that chloramphenicol, oxytetracycline, and chlortetracycline were effective in vitro. On the other hand, tests run in vivo with eastern brook trout (Salvelinus fontinalis) that had furunculosis or ulcer disease showed that chloramphenicol and oxytetracycline were effective, though chlortetracycline (aureomycin) had no therapeutic value (Snieszko 1954). Why the action of chlortetracycline in vitro differs so much from that in vivo is still to be explained.

The practical tests to determine the sensitivity of A. salmonicida to antibiotics can be made in any of the media used with sulfonamides. Single or multiple discs can be deposited on media in petri plates, or single discs on media slanted in test tubes. Antibiotics cannot, however, be added to media and sterilized in the autoclave. Commercial discs contain antibiotics in various concentrations. Discs with medium or high concentrations of antibiotics should be used in routine work.

TABLE 2.--Determination of susceptibility of Aeromonas salmonicida to some antibiotics as determined with Multidiscs and Leetown standard furunculosis medium

Antibiotic	Quantity of antibiotic in disc	Width of zone of growth inhibition (millimeters)
Chloromycetin[1/]	10 micrograms	14 - 19
Terramycin[2/] ---	10 do.	12 - 14
Tetracycline---	10 do.	10 - 13
Aureomycin[3/] ---	10 do.	10 - 12
Erythromycin---	10 do.	5 - 8
Streptomycin---	10 do.	4 - 6
Neomycin-------	10 do.	1 - 2
Penicillin-----	1.5 units	0 - 1
Carbomycin-----	5 micrograms	0
Bacitracin-----	5 units	0
Polymyxin B----	10 do.	0
Viomycin--[4/]---	10 micrograms	0
Furadantin[4/] ---	50 do.	8 - 10

[1/] Chloramphenicol. [3/] Chlortetracycline.
[2/] Oxytetracycline. [4/] Nitrofurantoin.

INTERPRETATION OF RESULTS

The results of sensitivity tests must be correctly interpreted. Caution must be used to avoid overestimating the significance of the in vitro tests for the selection of the most promising treatment.

It must be remembered that the sensitivity of a certain isolate of A. salmonicida, or other fish-pathogenic bacterium, to some antibiotics or sulfonamides in vitro does not necessarily mean that these drugs must be therapeutically effective in vivo. Some sulfonamides are poorly absorbed from the intestinal tract of fish, may arrest the growth of some species of trout, or may be toxic to treated fishes (Snieszko 1954; Wood, Yasutake, and Snieszko, 1955; Snieszko and Wood 1955). Thus, the drug-sensitivity test in vitro is of real value only if applied to drugs which are known to be effective in the treatment of fish diseases.

The in vitro drug-sensitivity test can also be of great value in screening new drugs for trial in treating fish diseases. If a particular fish pathogen is sensitive to

a particular drug in vitro, it is likely (but by no means certain) that this drug may be of value in treating a disease caused by the same bacterium. On the other hand, inactivity of a drug in vitro indicates that the therapeutic value of this drug is at least doubtful.

It is suggested that drug-sensitivity tests should be performed by persons who have basic knowledge of bacteriological or laboratory procedures and at hatcheries that have adequate facilities.

CONTROL OF FURUNCULOSIS

The general aspects of treatment of bacterial fish diseases have been reviewed elsewhere (Snieszko 1954). The most up-to-date treatment and preventive measures for the control of furunculosis may be listed as follows:

1. When there is an outbreak of a fish disease that is suspected of being furunculosis, specimens of moribund fish should be removed and treatment of the remaining fish with sulfonamides should be started immediately.

2. At the same time, bacteriological media should be inoculated with material removed from the kidney of moribund fish. Bacteriological examination should include the presumptive test as described by Griffin (1952) and determination of sensitivity to sulfonamides, oxytetracycline, and chloramphenicol. Also, specimens of moribund fish should be preserved in formalin and sent to the nearest fish-pathological laboratory.

3. If the presence of furunculosis is confirmed and the isolated cultures of A. salmonicida are resistant to sulfonamides, either chloramphenicol or oxytetracycline should be used. If tests show that the isolates are resistant also to one of the antibiotics, that one should not be used. If isolates are sensitive to both chloramphenicol and oxytetracycline, use of the former is considered somewhat better.

4. A number of sulfonamides can be used to treat furunculosis. On the basis of experimental evidence, sulfamerazine, a mixture of sulfamerazine with sulfaguanidine, and sulfamethazine are the best. As growth of brown trout is suppressed by sulfamerazine alone or in combination with other sulfonamides, sulfisoxazole (Gantrisin) should be tried with this species of trout (Snieszko and Wood 1955). Properties of this drug make it very promising, but there is not enough experimental evidence regarding its therapeutic value for fish.

5. The quantities of the sulfonamides used should be calculated on the basis of the weight of fish, not the weight or volume of feed. Sulfamerazine should be used at a rate of 8 to 10 grams (1/3 ounce) per 100 pounds of fish per day. If sulfamerazine with sulfaguanidine is used, fish should receive a mixture of 12 grams of sulfamerazine with 6 grams of sulfaguanidine for 100 pounds of fish per day during the first 3 days, followed by a 7-day regimen of 6 grams of sulfamerazine and 4 grams of sulfaguanidine daily. There is not enough experimental evidence to show which of the foregoing treatments is the better. In experiments with healthy trout, sulfamerazine alone (or with sulfaguanidine) had a depressing effect on growth of brown trout but had no significant effect on either brook or rainbow trout. Sulfisoxazole should be tried at the same rate as sulfamerazine. If mortalities are not brought to normal within 10 (at most, 20) days of treatment, some other treatment should be used. If sulfa-resistant cultures of A. salmonicida are isolated during the treatment, sulfonamides should be replaced immediately with antibiotics.

6. Chloramphenicol or oxytetracycline (terramycin) should be used at the rate of 2.5 grams (1/11 ounce) of pure antibiotic activity per 100 pounds of fish per day. If antibiotics are used in crude forms marketed as feed additives, the concentration of the pure antibiotic in the product should be determined and the amount used calculated accordingly. For example, if a product contains 25 grams of antibiotic per pound, it will require 0.1 pound of this material to furnish the 2.5 grams of pure antibiotic required. Antibiotics should not be purchased in a too-diluted form because the inert material may be harmful to fish or may replace too much

of the fish diet without supplying nutritional value. Antibiotics are more economical if purchased in the crude forms intended for livestock or poultry use.

7. Drugs should be used in the concentrations recommended. Using too large quantities may produce undesirable toxic effects and is uneconomical. Using too small quantities may cause only a partial reduction of mortalities, require a much longer period of treatment, or favor the appearance of drug resistance (Eagle and Saz 1955).

8. Drugs should be well mixed with the diet. If the diet is wet, it should be kept under refrigeration and should not be prepared for more than 3 days at a time. Sulfonamides can be added to pellets at the time of manufacture, but medicated pellets should be used in a reasonably short time (1 to 2 months) unless tests show that longer storage is practicable.

9. For the best results, treatment with sulfonamides should be followed immediately by a 3-day treatment with either chloramphenicol or terramycin, or vice versa. This may be expected to eliminate bacteria which might have developed drug resistance during the main course of treatment.

10. Treatment of furunculosis or of any other fish disease can be compared to the use of fire extinguishers in the control of fire. No one can deny the value of good extinguishers, but everyone considers prevention a better and a cheaper means of fire control. Similarly, prevention or prophylaxis is much more important as a means of disease control than treatment of a disease outbreak. Sanitation, selection and breeding, nutrition, proper hatchery practices, and prophylactic treatment (whenever applicable)--all should be used to prevent disease.

10-a. The purpose of sanitation is to prevent the pathogen from entering a hatchery or gaining a foothold. Excellent descriptions of hatchery sanitation were given by Davis (1953) and by O'Donnell (1947).

10-b. Equally important, if not more so, is the selection of disease-resistant trout. The importance of this approach was reviewed by Wolf (1954). Experiments at Leetown during the past several years have shown that strains of brook trout are very different in susceptibility to ulcer disease and furunculosis. Though susceptible strains can be destroyed by exposure to these diseases, the resistant strains have shown very slight or no losses (Snieszko, in press).

10-c. Nutrition is also very important. Animals suffering from malnutrition are believed to be more susceptible to bacterial diseases.

10-d. Hatchery practices such as cleanliness, grading, avoidance of crowding, and provision of proper water supply are of great importance.

10-e. Prophylactic treatments include disinfection of eggs with sulfamerthiolate or acriflavine, as well as periodic treatments with sulfonamides or antibiotics. Though egg disinfection is highly recommended, prophylaxis with sulfonamides or antibiotics may do more harm than good. Prophylaxis with sulfonamides would be too expensive to be practical; it would also promote the appearance of sulfa-resistant strains of A. salmonicida, necessitating treatment with the more expensive antibiotics. Use of antibiotics as fish-feed supplements has so far been found worthless (Snieszko 1957). If antibiotics were given in quantities sufficient to suppress fish-pathogenic bacteria, the cost would be prohibitive and there would be danger of the appearance of antibiotic-resistant strains of A. salmonicida (or other fish-pathogenic bacteria).

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APPENDIX

Composition of bacteriological media:

1. Leetown standard furunculosis medium:

Tryptic digest of casein (any make)----------grams--	10
Yeast extract, dehydrated (any make)----------grams--	5
Sodium chloride----------do.---	2.5
Agar----------do.---	15
Water----------liter--	1
pH----------	7.0 - 7.2

For sensitivity testing of sulfonamides, add 5 grams of secondary sodium phosphate and adjust the pH to 8.0. For motility testing of bacteria, use only 2 to 5 grams of agar. Omit agar for a liquid medium.

2. Mueller Hinton medium:

Beef, infusion from----grams--	300
Acid hydrolyzed casein--do.---	17.5
Starch----------do.---	1.5
Agar----------do.---	17
Water----------liter--	1
pH----------	7.4

3. Trypticase soy agar:

Trypticase----------grams--	15
Phyton (peptone from soy bean) grams--	5
Sodium chloride----------do.---	5
Agar----------do.---	15
Water----------liter--	1
pH----------	7.3

For the preparation of Mueller Hinton and trypticase soy media in liquid form,

the ingredients must be purchased separately and agar omitted from the formula.

It is very important that the treatment of diseases be started as soon as practicable, and that the drugs be used in proper quantities. Therefore, adequate quantities of the needed sulfonamides and antibiotics should be available to hatcheries without delay.

It is suggested that when large purchases of crude chloramphenicol or terramycin are made, samples may be sent to the Leetown laboratory for comparison of relative antibacterial activity with that of the pure form. Tests will be made in the simplest way with sensitivity discs and agar media. It is possible that such testing may be of value in determining the correct dosage of antibiotics in the crude form. Only crude forms of chloramphenicol or terramycin will be tested. Tests will be made if time and equipment are available.

Information regarding sources of supply for bacteriological media, sensitivity discs, antibiotics, and other materials may be obtained by writing to the authors.

AN EVEN-FLOW LIQUID TOXICANT DISPENSER

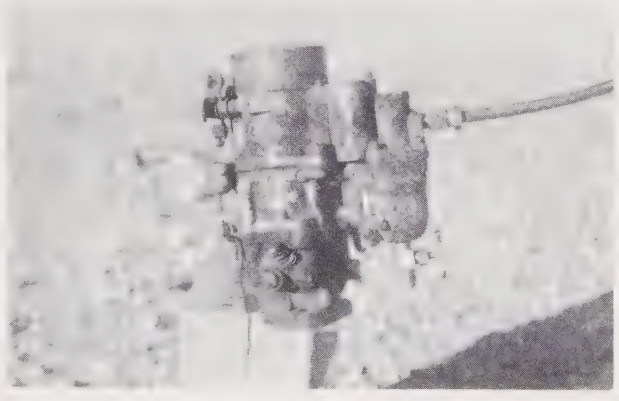
Rotenone-bearing liquid fish toxicants and potassium permanganate are used in some areas to control fish populations in streams with moderate or swift currents. The desired concentration of a toxicant is applied at one point long enough to get a complete kill. The kill is stopped at any desired point by dripping the right amount of potassium permanganate into the water for as long a time as the toxicant needs to move downstream.

Charles L. Sowards, fishery biologist of the Wyoming Game and Fish Department, points out, however, that for satisfactory results with this technique the liquids must be dispensed evenly. He recommends use of a device that has a wide range of discharges but provides a very constant flow after it is adjusted.

The dispenser that Mr. Sowards has found very effective has been made by connecting a large carburetor to a 30-gallon drum with the fittings and 30 inches of copper tubing (see photograph). A 5/16-inch straightway petcock has been inserted into a hole drilled and tapped into the bottom of the bowl of the carburetor. The needle valve and the float (the only parts in the carburetor that need to function) maintain a constant fluid level in the bowl, which in turn allows a constant flow from the petcock. Unless the barrel and the liquids to be discharged are extremely clean, an automotive fuel filter should be put between the barrel and the carburetor. The 6-inch head pressure required before the device will maintain a constant flow can be obtained by putting the carburetor 6 inches or more below the barrel.

"Two devices were tested," writes Mr. Sowards, "to determine how much the discharges fluctuated when head pressure was varied from 19 to 33 inches. With discharge petcocks set at about 100 milliliters per minute and using the average of 3 readings, the maximum variations were 5.6 and 1.9 percent, respectively; with petcocks wide open, maximum variations were 1.5 and 0.3 percent, respectively."

(Photograph courtesy of Mr. Sowards)



VARIATION IN STRENGTH OF PYRIDYLMERCURIC ACETATE TECHNICAL AND ITS EFFECT ON RAINBOW TROUT

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USE OF PYRIDYLMERCURIC ACETATE TECHNICAL (PMA) for treatment of various external diseases of fish, as suggested by Van Horn and Katz (1946) and tested first by Rucker (1948), has given excellent results in the past several years. It has been especially efficacious in the treatment of gill trouble among trout, although experiments by Rodgers, Hazen, Friddle, and Snieszko (1951) and the experience of others have proved its toxicity to rainbow trout (Salmo gairdneri). It would be of considerable advantage to the fish-culturist if this chemical could be used without danger of mortality among rainbow trout.

History of the Problem

In Michigan, PMA was employed successfully in controlling gill disease among brook trout (in dilutions up to 1:250,000) and among rainbow trout (at a dilution of 1:500,000), despite the fact that experiences in other States indicated that this chemical could be lethal to rainbow trout. After PMA had been used about 2 years in treating gill disease among rainbow trout, there were two incidents in which a high mortality followed treatments. The fact that PMA from two sources was involved suggested that the chemical from only one of the sources might be lethal to the fish.

The first incident occurred at the Oden hatchery, where raceways are constructed in two parallel series of five ponds each. The upper four ponds of each series contained brook trout, and the lowest pond of each series held legal-sized rainbow trout. During the morning of October 11, 1954, brook trout in the uppermost pond of each series were treated with PMA and the treated water was flushed through the lower four ponds as usual. By chance, the chemicals used in the two series were from different chemical companies. On October 11 and 12, a 7-percent mortality occurred among the 8,000 rainbow trout in one of the lowest ponds; rainbow trout in the comparable pond of the other series were not adversely affected. As fish were killed in one pond and not in the other, there were two possible causes for the mortality: either there had been an error in making the stock solution for treating one series of ponds, or the PMA from one of the sources was at fault. Later tests proved that error in making the stock solution had not been responsible for the loss.

The second incident took place at the Harrietta hatchery on March 3, 1955, when rainbow trout fry were given the usual prophylactic treatment with PMA. Several hours later there began a mortality which extended throughout the following day and resulted in the loss of about 600,000 fry. The PMA employed for this treatment had been received from a source different from that of the PMA previously used at

Harrietta and was identical to the PMA presumed to have caused the loss of legal-sized rainbow trout at the Oden hatchery. On March 4, in an attempt to establish the cause of the previous day's loss of fry, Mr. J. Southwick, the hatchery superintendent, treated one group of rainbow trout fry with PMA from one source and another group with PMA from the second source. Fish exposed to the PMA which had previously been employed at Harrietta (here termed No. 1) remained normal, but all fish in the group treated with PMA from the new source (No. 2) died.

In view of the incidents just described, experiments were conducted in aquaria to test the chemicals from the two sources under controlled conditions.

Effect of PMA From Two Sources on Brook Trout and Rainbow Trout

For each of several tests 10 fish were placed in an aerated aquarium and exposed for 1 hour to a dilution of PMA. Tests with each of several dilutions were made in duplicate. At the end of the hour, the treated water was replaced with fresh water and the fish were observed for 16 hours. Water temperature was 55° F. The results were as follows:

PMA No. 1

Rainbow trout:	
1:500,000	-- No mortality in 16 hours.
Brook trout:	
1:500,000	-- No mortality in 16 hours.

PMA No. 2

Rainbow trout:	
1:500,000	-- All 20 fish died in 16 hours.
1:1,000,000	-- Twelve of 20 fish died in 16 hours.
Brook trout:	
1:500,000	-- No mortality in 16 hours.
1:250,000	-- No mortality in 16 nours.

Mortality occurred between 2 and 8 hours after transfer of the fish from the treated to fresh water.

The results of this experiment were reported to the Mallinckrodt Chemical Works in St. Louis, Missouri, and a sample of PMA from each source was sent to them for analysis. They reported that sample No. 1 assayed 81.4 percent PMA and sample No. 2 assayed 87.0 percent PMA. They also indicated that the composition of Pyridylmercuric Acetate Technical varies to some extent. Because it is used principally as a fungicide for industrial applications, a pure product is not required; nor, for the intended applications, is it necessary to identify or regulate closely the percentages of various impurities contained in the compound. The assay values reported for this product are actually based on the mercury content of the material and do not necessarily represent the pyridylmercuric acetate content as such. The reported values include all other organic mercury compounds which might be present, such as polymercurated pyridine compounds, and these would be reported as an equivalent amount of pyridylmercuric acetate. Also, the product is reported to contain varying proportions of alkali acetate, which could cause significant differences in assay from lot to lot.

Effect of PMA of Different Composition on Rainbow Trout Fingerlings

Upon request, the Mallinckrodt Chemical Works kindly furnished 4 samples of PMA which assayed 81.8 percent, 84.4 percent, 84.8 percent, and 87.3 percent. Each of these 4 samples was tested on fish in dilutions of 1:400,000, 1:500,000, and 1:600,000; and each dilution was tested on 3 lots of 10 fish each. Only rainbow trout fingerlings were subjected to the tests, because earlier results had demonstrated that the brook trout were not adversely affected by PMA from either source.

Among those lots of fish exposed to samples of PMA assaying 81.8 percent, 84.4 percent, and 84.8 percent, there was no mortality in 24 hours after treatment. Among the lots exposed to the 87.3-percent sample, mortality within 24 hours after the treatment was as follows:

FIGURE 1.--Section of gill of rainbow trout 30 minutes after 1-hour treatment in PMA at 1:400,000. Note slight thickening at tip.

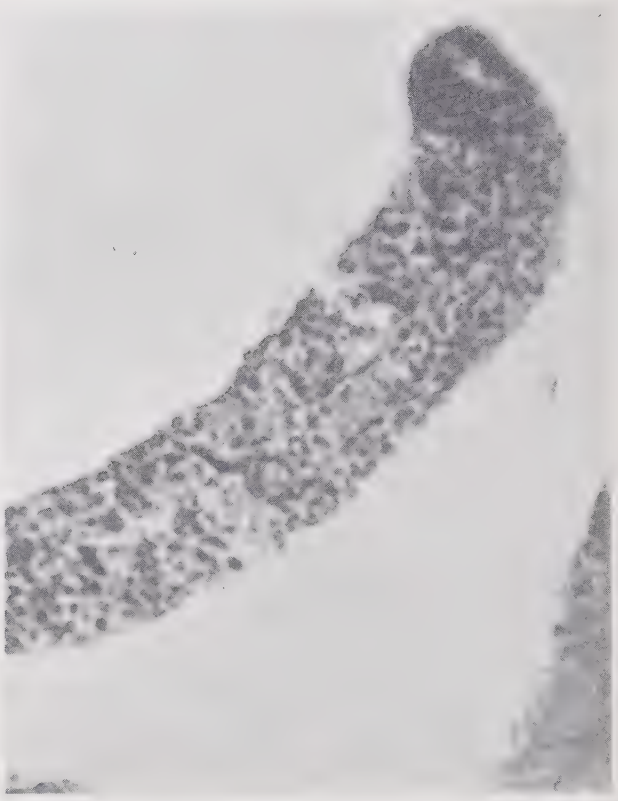

FIGURE 2.--Section of gill of rainbow trout 5 hours after 1-hour immersion in PMA at 1:400,000. Note that filament is affected for entire length.

(Photographs courtesy of the author)

at the 1:400,000 dilution, 24 of 30 fish died; at the 1:500,000 dilution, 20 of 30 fish died; and at the 1:600,000 dilution, 11 of 30 fish died.

Discussion

Because of the variations in impurities contained in Pyridylmercuric Acetate Technical, the results of the experiments were not clear-cut. For instance, there was no mortality when the 81.8-percent sample of PMA was tested in a dilution of 1:400,000 with a relative mercury concentration of 2.04. However, 11 of 30 fish died when the 87.3-percent sample was tested in a dilution of 1:600,000 with a relative mercury concentration of only 1.46. Obviously, the 87.3-percent sample contained an impurity that was toxic to rainbow trout.

As the impurities in PMA are said to vary from one batch to another, it is reasonable to assume that a toxic substance might also be found at times in the product assaying 81 percent. Therefore, aquarium tests should be made on each new batch of PMA before large numbers of rainbow trout are treated.

At present this information is of little value for practical application in treating rainbow trout, because there is not sufficient demand to make it profitable for any company to produce pyridylmercuric acetate within the percentage and composition limits that are safe for rainbow trout. However, it is always possible that such a demand may develop in the future.

The fact that, in all cases, mortality did not begin until about 2 hours after treatment suggests that an antidote might be given immediately after treatment. Because no substitute for PMA has been found that is as efficient in controlling gill disease, a search for an antidote should be profitable. Microscopical study of sections of gills taken at intervals up to 5 hours after treatment demonstrated progressive deterioration of the tissue (figures 1 and 2), and in some stages fusion occurred as it appears in gill disease. An antidote given soon after exposure to PMA might arrest this action on the gills and prevent mortality.

In conducting experiments with PMA on rainbow trout, fish that have never been exposed to this chemical should be used for each test because of the possibility that a tolerance may be developed. This possibility is suggested by the method formerly used at Harrietta for weekly prophylactic treatment of rainbow fingerlings. Personnel at Harrietta found that mortality could be avoided if the first few treatments were made at a dilution of 1:600,000; thereafter the dilution was gradually reduced until it reached about 1:400,000.

Acknowledgments

Thanks are due to the Mallinckrodt Chemical Works of St. Louis, Missouri, for assaying samples of pyridylmercuric acetate sent to them and for supplying PMA of specific percentages for these experiments.

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NEW USE FOR SALT BEING INVESTIGATED

In future, common salt may be used to measure stream volumes and velocities, to reduce excessively high electric resistivities of streams, and to increase overall efficiency of electrofishing during the cold months.

In the last quarter of 1956, biologists started experiments with cattle salt on streams in the Shenandoah and the Great Smoky Mountains National Parks. Using 50-pound blocks of the salt, they easily determined water volumes and velocities over distances ranging from 100 yards to 2.1 miles. Also, the blocks lowered the resistivities of selected streams by as much as 82 percent. As blocks of various manufacture lasted about 4 hours, the cost of application was about 20 cents an hour.

"Tests with salt are continuing," reports Robert E. Lennon, in charge of Eastern Federal Waters Investigations for the U. S. Fish and Wildlife Service, "but early results indicate that the efficiency of electrofishing operations is greatly increased. The effective field of the electrode system is extended, and the fish are more completely stunned and easier to pick up. A number of comparative collections made in salted and salt-free waters show that more of the available fish are taken on the first pass through a given area when the salt is used."

A VITAMIN TEST DIET FOR LONG-TERM FEEDING STUDIES

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U. S. Fish and Wildlife Service
Salmon Nutrition Laboratory, Cook, Washington

A NUMBER OF TEST DIETS are available for short-term feeding studies with trout as the experimental animal. McCay and Dilley (1927) postulated the requirement of trout for a factor H present in fresh meats. Rapid advancements in research showed that certain vitamins were required (McCay et al. 1927, Titcomb et al. 1929, Tunison et al. 1942, Wolf 1942, Woodbury 1943) and that under certain conditions anemia was prevented by the use of xanthopterin (Simmons and Norris 1941) or mixtures of B vitamins (Tunison et al. 1943). McLaren, Keller, O'Donnell, and Elvehjem (1947a) described some of the water-soluble vitamin requirements for rainbow trout (Salmo gairdneri), using a diet consisting of commercial casein, starch, fish liver oils, crab meal, minerals, and vitamins; but extended feeding trials with this diet and rainbow trout and feeding trials with chinook salmon (Oncorhynchus tshawytscha) were unsatisfactory (Rucker et al. 1952). Wolf (1951) developed a satisfactory vitamin test diet for rainbow trout by feeding commercial casein, potato starch, gelatin, minerals, hydrogenated cottonseed oil, cod-liver oil, and crystalline vitamins. He was able to describe requirements of rainbow trout for pantothenic acid, inositol, and folic acid; but groups of fish on diets deficient in niacin, biotin, ascorbic acid, vitamin B_{12}, and vitamin E failed to develop recognized deficiency syndromes (Wolf 1951).

Subsequent work with this diet and rainbow trout, brown trout (Salmo trutta), and brook trout (Salvelinus fontinalis) led to a confirmation of the growth responses reported by Wolf in 1951 (Phillips et al. 1952, 1953, 1954, 1955): but no extensive long-term feeding trials had been attempted with rainbow trout. Halver (in press) altered Wolf's diet in certain components and developed specific water-soluble vitamin-deficiency syndromes in chinook salmon. It seemed logical, therefore, to conduct an extended, long-term feeding trial with rainbow trout to test the adequacy of this diet for possible fat-soluble or reproductive vitamin studies.

Experimental Work

The vitamin test diet was formulated (Halver, in press) from the materials listed in table 1. Prior to the preparation of the diet, the crystalline vitamin supplement, the amino acid supplement, and the alpha-cellulose flour were mixed thoroughly in a ball mill and were then refrigerated at 41° to 50° F. until use. To insure the use of more accurate and reproducible weights, sufficient amounts of this mixture for at least 4 kilograms of diet were made ready at one time. The diet was prepared by suspending the solid ingredients in a warm gelatin solution and

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The present address of Mr. Coates is: State of Washington Department of Fisheries, Seattle, Washington.--The EDITORS.

TABLE 1.--Composition of the vitamin test diet

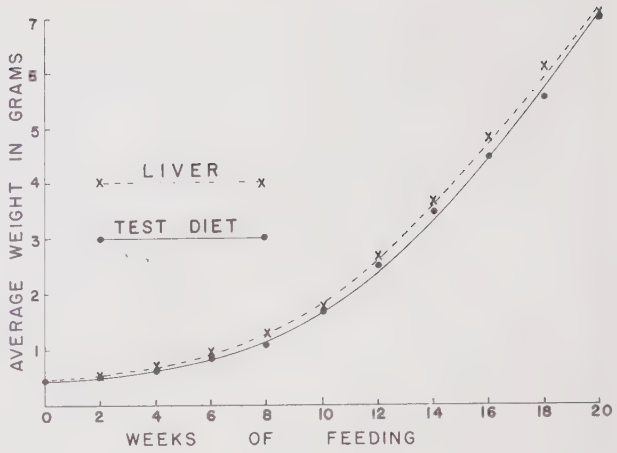
Main mixture	Parts	Mineral mixture	Amounts	Vitamin supplement	Amount[1]
			Grams		Milligrams
Vitamin-free casein--	54.0	U.S.P. XII Salt mixture No. 2--	100		
Gelatin, purified----	15.0			Thiamine hydrochloride----------	6.000
Corn oil, purified---	7.0			Riboflavin----------	20.000
Cod-liver oil--------	2.0		Milligrams	Pyridoxine hydrochloride---------	4.000
White dextrin--------	8.0			Nicotinic acid----------	80.000
α-Cellulose flour----	9.0	Aluminum chloride----------	18	Calcium pantothenate----------	28.000
Mineral mixture------	4.0	Zinc sulfate----------	357	Inositol----------	400.000
DL-Methionine--------	1.0	Cuprous chloride----------	11	Biotin----------	.600
L-Tryptophan---------	.5	Manganous sulfate----------	80	Folic acid----------	1.500
Crystalline vitamin supplement		Potassium iodide----------	17	p-Aminobenzoic acid----------	40.000
		Cobaltous chloride----------	105	Choline chloride----------	800.000
				Ascorbic acid----------	200.000
				α-Tocopherol----------	40.000
				Menadione----------	4.000
				Crystalline vitamin B_{12} (Merck)--	.009

[1] These amounts of individual vitamins were added per 100 grams of the main mixture.

blending thoroughly in a mechanical mixer during the cooling period.

Details of diet preparation and methods of feeding were identical with those previously described (Halver, in press). After being prepared, the ration was stored in screw-top glass jars and refrigerated until needed. Only as much diet as was necessary for a week of feeding was prepared at one time. For control purposes during the first 20 weeks of feeding, fresh ground liver was obtained from the Experimental Hatchery of the School of Fisheries, University of Washington, for the control lots.

Five thousand rainbow trout fry were obtained from brood stock of the University of Washington's School of Fisheries, were placed in a 10- by 12- by 168-inch screened hatchery trough equipped with a dechlorinated water supply from Seattle, and were taught to feed on finely ground beef liver. After 10 days the fry were feeding actively on the diet. Two lots of 1,000 fish each were transferred to individual troughs and were fed the complete test diet. Two additional lots of 1,000 fish each were fed the beef liver diet as a control. For the first 20 weeks of feeding, the fish were fed 3 times daily, 6 days weekly with no diet fed on Sundays. Troughs were cleaned daily to prevent any accumulation of fecal matter, and dead fish were removed as soon as they were observed. The individual groups of fish were weighed biweekly by removing the entire population in a net and weighing on a solution balance by the methods previously described (Halver, in press). Samples were removed periodically for the examination of external and internal organs to detect the appearance of any vitamin-deficiency syndromes reported by McLaren (1947b). After 12 weeks of feeding, all populations were reduced to 500 fish per trough to prevent overcrowding. After 20 weeks of feeding, cursory examinations failed to disclose any recognizable vitamin-deficiency syndromes and the two lots on the complete test diet were combined in the interest of space economy in the hatchery (see figure and table 2). At this time the liver-control lots were discontinued.



A comparison of the growth of rainbow trout fed freshly ground beef liver and rainbow trout fed the complete vitamin test diet.

The lot receiving the complete test diet was placed in a 6-foot circular tank, the population was reduced to 200 trout, and the number of feedings was reduced to 2 a day, 6 days weekly with no diet fed on Sundays. After 25 weeks of feeding, it was necessary to reduce the population to 100 fish in order to hold them conveniently in the circular tank. After 1 year of feeding the complete test diet, the fish had reached 150 grams in weight and a 5-fish sample was removed for thorough external and internal examination. After 57 weeks of feeding, the water temperature rose above 65° F. and it was necessary to refrigerate the water supply to prevent possible adverse environmental effects. After 5 weeks of refrigeration, the temperature of the water dropped and it was possible to discontinue cooling and to transfer the fish to an outside raceway that received the same dechlorinated city water supply.

The feeding program continued for an additional year, at the end of which time the fish averaged 1,100 grams (about 2.4 pounds). Eggs were removed from the mature females after 2 years on the diet and were fertilized with 2 ripe males each. The progeny from 1 female were kept on a single tray, and infertile eggs were removed periodically to prevent the growth

TABLE 2.--Summary of growth, mortality, and food conversion [1]

Ration	Initial weight	Final weight	Percent gain	Weight gain	Mortality	Percent mortality	Food fed [2]	Conversion
	Grams	Grams		Grams			Kilograms	
Complete test diet-------	0.39	6.90	1670	3,140	23	4.6	19.5	6.4
Complete test diet-------	.39	6.85	1655	3,130	20	4.0	19.5	6.2
Liver--------	.39	7.02	1700	3,140	20	4.0	15.3	4.9
Liver--------	.39	7.25	1755	3,280	26	5.2	15.3	4.7

[1] Calculated on basis of 500 fish per lot.
[2] Calculated on basis of wet diet fed (25 percent solids).

of fungus. After the fish had spawned, they were continued on the diet for an additional year. In the interim they were transferred to the Salmon Nutrition Laboratory at Cook, Washington, and a new water supply with different temperature characteristics. As soon as the yolk sacs had been absorbed in the progeny from the initial spawning, these fish were also placed on the complete test diet and continued for a year on the new water system.

Results and Discussion

The growth response of rainbow trout on the complete test diet compared favorably with that of the rainbow trout on the liver control diet after approximately 8 weeks of feeding. The initial lag in the growth rate may have been due to the change in diet or to the protein content of the complete test diet, as recent information indicates that the protein intake of fish may be directly proportional to the water temperature (DeLong et al., in press). After the fish had been on the diet 8 to 10 weeks, the water temperature rose above 50° F. and the fish fed actively on the 70-percent protein diet. A direct comparison of the growth rate of the fish on the complete test diet with the growth rate of those on the hatchery production diet at the 1-, 2-, and 3-year feeding intervals was not possible because the liver-controlled lots were discontinued after 20 weeks of feeding. A comparison of average weights with the average weight of fish found in the School of Fisheries' production-diet brood stock (Donaldson and Olson, in press) indicated that the rainbow trout on the complete test diet were approximately 10 percent lighter than those on the production diet. One must consider, however, that the production-diet fish were confined in a larger raceway and had been in the Lake Washington water, which had a different temperature pattern and probable environmental characteristics.

After the brood stock had been transferred to the colder water system (47° F.) at the Salmon Nutrition Laboratory and there confined in a 12-foot circular tank, the fish did not grow to any great extent; they merely increased their weight by approximately 20 percent during the year. They did spawn the following year, however, and the eggs were viable. At the end of 2 years on the complete test diet, the fish averaged 1,100 grams (about 2.4 pounds) and spawning commenced early in the spring. Nineteen females matured over an extended period from March 7 to May 20, 20 males with active sperm were available for fertilization of eggs, and 3 fish tentatively classified as females did not mature. Egg production seemed normal with an average of approximately 2,000 eggs produced per female. Approximately 38,000 eggs were obtained from 19 females, and the total loss to the actively feeding fry stage was 33.3 percent. This

loss could be broken down into: (1) Unfertilized loss, approximately 5 percent; (2) loss at the eyed egg stage after shocking, approximately 10 percent; and (3) loss of approximately 15 to 20 percent on hatching and yolk-sac absorption. From 38,000 eggs it was possible to obtain approximately 25,000 actively feeding fry, which indicated that the total viability of the eggs produced was approximately 66.6 percent. This value, although low, corresponds favorably with the viability percentage on the eggs obtained at the School of Fisheries' Experimental Hatchery from the brood stock during the spring and summer of 1953.[1/]

As soon as the yolk sacs had been absorbed, the fry were put on the complete test diet and were transferred to the new experimental hatchery in the Salmon Nutrition Laboratory at Cook, Washington. These fry were fed for an additional 18 months on the complete test diet without the appearance of any recognizable vitamin-deficiency syndromes. At the cold water temperatures (38° to 42° F.), growth was severely curtailed; and after 18 months on the diet the fish weighed 110 to 150 grams, though their parent stock in the same time interval and in different water temperatures averaged well over 600 grams (about 1.3 pounds). During the following year, in the spring of 1954, 9 of the original females spawned for the second time (100 percent). From this group there were obtained approximately 19,000 eggs, from which 13,500 actively feeding fry developed. The viability percentage on the second spawning was 71.5 percent to the advanced fry stage, or a total loss of approximately 28 percent. The parent stock were kept on the complete test diet an additional 6 months and were finally used for studies in which the exact nutritional background of the fish was desirable. These fish were then sacrificed over a period of about 3 months for various biological determinations not connected with this experiment.

[1/]Personal communication from Dr. L. R. Donaldson, School of Fisheries, University of Washington, Seattle, Washington.

The progeny from the second spawning were held in the cold water temperatures until the yolk sacs had been absorbed and the fry were feeding actively on the complete test diet. The fish were held an additional 20 weeks on the complete test diet and were planted. During this continued feeding program, no recognizable vitamin-deficiency syndromes were observed. The fish fed actively, consumed the diet within a very short time after its presentation, and exhibited general characteristics of good health. Undoubtedly warmer water would have resulted in more effective growth during the extended feeding periods. It is possible that the protein content of the complete test diet in this cold water was excessive, resulting in an internal uremia or toxemia and inhibition of the nitrogen metabolism in the animal at this temperature.

Costs

The cost of the ration has lowered each year as reductions in the cost of the purified ingredients and crystalline vitamins have occurred. By purchase of reasonably large quantities of material at the present prices, this diet could be prepared for $0.755 per pound. This cost could be broken down as follows: Vitamin-free basal ration, $0.35; amino acid supplement, $0.305; and crystalline vitamin supplement, $0.10.

Conclusions

A group of rainbow trout were held through two reproductive cycles on the complete test diet without the appearance of any recognizable vitamin-deficiency syndromes.

Egg production and viability of the test group were normal and compared favorably with those of the parent brood stock during the same period.

The first spawning resulted in 66.6 percent viable, actively feeding fry from the females on hand.

In 2 years of feeding, 87 percent of the females and 100 percent of the males matured.

It was demonstrated that the complete vitamin test diet was satisfactory for extended feeding trials with rainbow trout for a period of at least 3 years.

Use of this diet for fat-soluble vitamin studies or biological investigations where the specific nutritional background of the fish is desirable in terms of the basic nutrients, is now possible.

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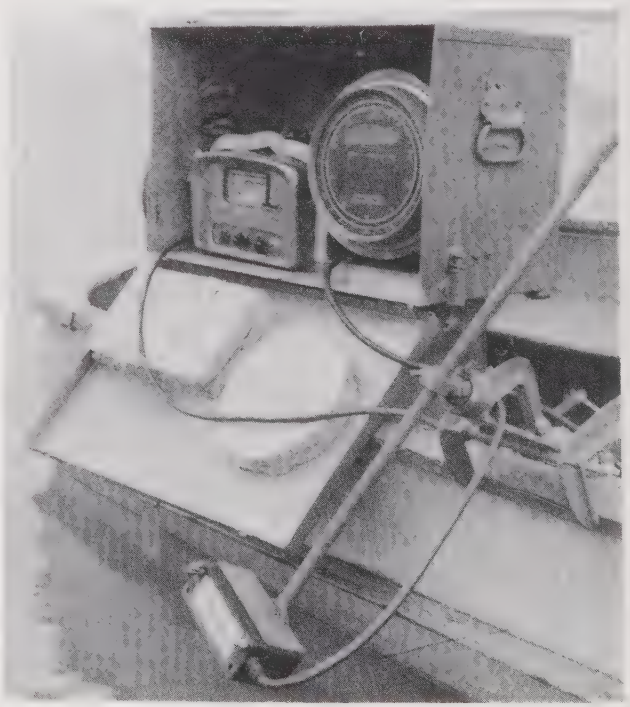
THE MOUNTING OF ECHO-SOUNDING EQUIPMENT

As echo-sounding units of the Wyoming Game and Fish Department must be available for use in various parts of the State, it has been necessary to have the equipment mounted for safe, convenient transfers over considerable distances in pickup trucks. Lawrence W. Peterson, fishery biologist, reports that the mounting shown in the photograph offers ease of handling and protection during shipment.

The depth indicator and recorder are mounted in sponge rubber in an army-surplus metal box (about 13 by 15 by 24 inches). Pads of sponge rubber glued to the lid protect the faces of these instruments when the box is closed. Door stops that are mounted on the front sides of the box allow the operator to raise its front edge and get a better viewing angle. The transducer-to-boat mounting is a hinged plate set between two large C-clamps. When the boat is nearing shore or moving fast, the operator can pull the support rod of the transducer crosswise to the boat, thus lifting the transducer from the water and reducing undue strain on the mounting. An auto-starter spring attached to the hinged plate is rotated so that the support rod is pointed slightly forward when the boat is not moving. Water pressure puts a tension on the spring to keep the rod in a vertical plane when the boat is moving. If a solid object strikes the transducer or rod, the spring allows the rod to bend backward far enough to clear this object. A set screw is used to adjust the support rod up or down.



(Photograph courtesy of Mr. Peterson)

THE NUTRITION OF TROUT
IV. VITAMIN REQUIREMENTS

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and
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VITAMINS are substances needed by all forms of life (both plant and animal) for existence. The vitamins are considered catalytic in nature, as they produce or accelerate chemical reactions without taking part in those reactions. It is on the basis of their action within the body, rather than because of chemical relationship, that vitamins are grouped under one heading. Actually the chemical relationship of the vitamins is not close.

Vitamins were discovered early in the present century and they were thought to be amines vital for the existence of life. From the term "vital amines" comes the name "vitamin." Later studies have shown that vitamins are not related amines but are compounds that have similar, although specific, functions within the body.

The amounts of vitamins required by the body are very small. Often the daily requirement is too small for weighing on even the most sensitive chemical balance.

For convenience, vitamins are broadly classified as (1) fat-soluble vitamins and (2) water-soluble vitamins. The first group includes the vitamins that are dissolved by fat solvents such as ether and alcohol. These vitamins are usually found associated with natural oils and fats, such as cod-liver oil, wheat germ, and butter. The second group includes the vitamins that are dissolved by water. Beef liver, yeast, cereals, and fruits and vegetables are sources of these vitamins.

In general, vitamins are widely distributed in foods, but some foods contain greater amounts than others. Cod-liver oil is an excellent source of both vitamins A and D, whereas wheat flour is a poor source of these vitamins. Wheat and yeast are rich in some water-soluble vitamins, but cod-liver oil contains none of them. Foods that are high in vitamin content are called concentrates. Some of these vitamin concentrates occur naturally (yeast), and some are commercially prepared. The concentrates are prescribed for patients suffering from vitamin deficiencies.

The vitamin requirements have been established for a number of the higher animals. The application of this knowledge has improved the health of the animals and has reduced the cost of rearing by allowing the use of low-grade foods that have been fortified with the necessary vitamins.

VITAMINS IN THE DIET OF TROUT

Until recently, specific studies of the vitamin requirements of trout were impossible because a diet that was composed of chemically pure substances and that would maintain trout life over long periods was not available. In 1951 Wolf described a synthetic diet that would maintain trout. As all of the ingredients, including the vitamins, were chemically pure, it was possible to withdraw the vitamins one at a time and determine the necessity for their presence for growth and survival of trout. Wolf's diet represents one of the finest

contributions to trout-nutrition studies reported during the last 20 years. The application of Wolf's diet has resulted in the determination of the necessity for 10 members of the vitamin B complex for trout.

In addition to the studies made with Wolf's synthetic diet, there have been studies which attempted to establish the minimum daily requirement of trout for some of the vitamins. These studies have been based on the fact that limited amounts of members of the vitamin B complex are stored in the body, while excess dietary levels are excreted. By feeding different combinations of foods--combinations that contain different levels of the vitamins--it was possible to determine the dietary levels that caused maximum storage of the vitamins within the body. Because these requirements have been established by varying dietary ingredients (usually liver and spleen) and because the requirements are based upon storage alone, they are called tentative daily requirements for the fish.

The two methods just described have made possible an intensive study of the vitamin B complex requirement of trout. Less progress has been made with the requirements for the fat-soluble vitamins, and today little is known of the effect of this group of vitamins on the health and survival of trout.

The Fat-Soluble Vitamins

Of the four fat-soluble vitamins (D, E, K, and A), only vitamin A has been reported as essential for trout. A deficiency of vitamin A has been reported as the cause of cataract in trout. Other studies, however, have failed to confirm the need of trout for vitamin A. Cod-liver oil has been reported to stimulate the growth of trout by reason of its high content of vitamin D. Recent studies, however, have shown that although cod-liver oil does increase the growth rate of trout, neither vitamin A nor vitamin D is responsible for the increased growth of the trout. Nevertheless, until it has been definitely proved otherwise, it is safe to assume that all of the fat-soluble vitamins are necessary for trout.

The Water-Soluble Vitamins

Vitamin B_1, or Thiamine.--Vitamin B_1 was the first vitamin reported essential for trout. A deficiency of B_1 in the diet of the fish causes nervousness, a retracted head, and--at times--a purplish sheen on the body. A sudden movement near the pond of trout deficient in B_1 will cause large numbers of the fish to turn on their sides, and many of these fish will fail to recover. Large mortalities accompany a deficiency of B_1. A deficiency of vitamin B_1 may be caused by using fresh fish in the diet of trout. If fresh fish--carp, goldfish, suckers, and herring are a few of the species used--is allowed to stand with other diet ingredients, a deficiency of B_1 will result when the food is given to trout. Vitamin B_1 is essential for brook, brown, and rainbow trout.

The tentative daily requirement has been established as between 0.150 and 0.186 milligram per kilogram of trout body weight per day.

Beef liver, dried brewer's yeast, wheat middlings, dried skim milk, and distiller's solubles are sources of vitamin B_1.

Vitamin B_2, or Riboflavin. --A deficiency of vitamin B_2 in brook and brown trout causes an almost complete stoppage of growth. Lake trout that are deficient in vitamin B_2 also develop an opaqueness of the eye. It is possible that this opaqueness of the eye would develop in both brook and brown trout if the deficiency were continued for extended periods. No experiments on the requirement of rainbow trout for vitamin B_2 have been reported.

The tentative daily requirement has been established as between 0.44 and 0.68 milligram per kilogram of body weight per day.

Vitamin B_2 is found in beef liver, dried brewer's yeast, distiller's solubles, dried skim milk, and various cereal products.

Vitamin B_6, or Pyridoxine. --A deficiency of vitamin B_6 will cause complete mortality among brook, brown, and lake

trout in a period of 6 to 12 weeks. No experiments for rainbow trout have been reported, although it is safe to assume that these trout would also require B_6.

The tentative daily requirement has been established as between 0.225 and 0.250 milligram per kilogram of trout body weight per day.

Vitamin B_6 is found in beef liver, dried brewer's yeast, distiller's solubles, and various cereal products.

Vitamin B_{12}. --A deficiency of vitamin B_{12} causes a reduction in the rate of growth of brown trout. No need for B_{12} has been established for brook, rainbow, or lake trout. It is doubtful that there is a species difference in the need for vitamin B_{12}; it is more likely that impurities in the synthetic diet satisfy the need of brook, rainbow, and lake trout but fail to do so for brown trout.

No tentative daily requirement has been reported for this vitamin.

Vitamin B_{12} is found mostly in beef liver and cereal products.

Biotin. --A deficiency of biotin in the diet of brook, brown, and lake trout reduces the rate of growth of the fish. Wolf has reported that rainbow trout do not need biotin. In brook, brown, and lake trout, biotin deficiency has caused "blue-slime" or "slime-patch" disease.

The tentative daily requirement has been established as between 0.0433 and 0.0796 milligram per kilogram of weight per day for brown trout, and between 0.00678 and 0.0268 milligram per kilogram of body weight per day for brook trout.

The best sources of biotin for trout diets are beef liver and dried brewer's yeast. Because of the relatively low levels of biotin in most foods, trout diets should be fortified with either beef liver (20 percent) or dried brewer's yeast (5 percent) to assure the presence of adequate levels of dietary biotin.

Vitamin C, or Ascorbic Acid. --Vitamin C is not considered essential for trout. Although there have been reports that vitamin C is needed by trout, additional studies have failed to confirm this reported need.

Choline. --A lack of choline in the diet of brook and brown trout reduces the rate of growth of the fish. Choline has not been found essential for lake trout, and no results have been reported for rainbow trout.

No tentative daily requirement has been established.

Choline is abundant in the foods of hatchery fish, and a deficiency is unlikely. Beef liver, dried brewer's yeast, distiller's solubles, and most cereal products contain high levels of choline.

Factor H. --Many years ago it was determined that fresh meat was necessary in the diet of trout, and that they became anemic when meat was eliminated from the diet. The substance in fresh meat that prevents anemia is known as factor H. The nature of factor H remains unknown, and many workers believe that more than one vitamin is involved. The term "anti-anemic factors" is probably more accurate than the term "factor H." Fresh meat must be supplied in the diet of trout to prevent anemia.

Folic Acid. --A deficiency of folic acid reduces the growth rate of brook, brown, and rainbow trout. It is not known to be essential for lake trout. Of the four species of trout, only for the rainbow trout has a folic acid deficiency been described as a cause of anemia.

The tentative daily requirement has been established as 0.00292 milligram per kilogram of body weight per day.

The richest sources of folic acid are beef liver and dried brewer's yeast.

Inositol. --Inositol is a necessary growth factor for brook, brown, and rainbow trout. It is not known to be essential for lake trout. No deficiency symptom other than reduced growth has been described for brook and brown trout, but an inositol deficiency has been reported as a cause of anemia in rainbow trout.

No tentative daily requirement has been established.

Inositol is found in beef liver, dried brewer's yeast, distiller's solubles, and various cereal products.

Vitamin P-P, or Niacin. --Niacin is an essential growth factor for brook and

brown trout but is not known to be essential for rainbow and lake trout.

The tentative daily requirement has been established as between 3.0 and 4.1 milligrams per kilogram of body weight per day.

Niacin is found at relatively high levels in beef liver, dried brewer's yeast, and distiller's solubles, and some cereal products.

Pantothenic Acid. --Pantothenic acid is essential for brook, brown, rainbow, and lake trout. Trout that are deficient in pantothenic acid develop a severe gill disease characterized by swollen and proliferated gill tissues. High mortalities result. A deficiency in pantothenic acid has been reported as the cause of "blue-slime" disease of trout. It is probable that both pantothenic acid and biotin are causes of "blue-slime" disease.

The tentative daily requirement has been established as between 0.97 and 1.25 milligrams per kilogram of body weight per day.

Good sources of pantothenic acid are beef liver, dried brewer's yeast, dried skim milk, and distiller's solubles. Of all the vitamin deficiencies, a deficiency in pantothenic acid is probably the most common among trout.

GENERAL COMMENTS

With the present knowledge of the vitamin requirements of trout, it is possible to compound relatively sound trout diets. Many of the diseases of hatchery trout that are attributed to unknown causes--and the severity of the attack of some of the bacterial diseases--may be caused by or exaggerated by vitamin deficiencies. It is essential that every precaution be taken to fill the known vitamin needs of the fish in order to preclude as many of the nutritional difficulties as possible. Of the 10 vitamins known to be essential for trout, only 4 (thiamine, pantothenic acid, biotin, and riboflavin) have recognizable, specific, external deficiency symptoms. The need for the other six vitamins has been established by their effect on the growth rate of the trout. As previously pointed out, growth rate is not a specific function for most of the vitamins. The vitamins that show an effect on only the growth and mortality of trout (choline, folic acid, B_{12}, niacin, inositol, and pyridoxine) have specific effects in the bodies of the higher animals. It is reasonable to believe that these six vitamins also have specific (but at present unknown) effects in the trout body. In general, the B-complex requirement of trout may be filled by the inclusion of either 20 percent beef liver or 5 percent dried brewer's yeast in the diet.

Although species differences for trout have been reported in the literature, it is safe to assume that all of the vitamins are required by all four species of trout (brook, brown, rainbow, and lake trout). Thus, although recent studies have shown that lake trout require only 4 of the 10 vitamins necessary for brown trout, these lake trout experiments were conducted at relatively low water temperatures for lake trout, and it is probable that the cold water--and the slow growth rate of the fish--prevented the appearance of deficiency symptoms. It is unlikely that there are true species differences between such closely related fish species.

If the dietary vitamins are to serve the trout body, the food must be handled in a manner assuring the preservation of the vitamins until the food is eaten. Freezing is the best means of preserving vitamins in meat products. Dry meals should be stored in a cool, dry area. Food should not be mixed and allowed to stand. Even at relatively cool temperatures, oxidation may destroy vitamins or the ingredients may interact and destroy essential elements. It is best to mix foods daily; if this is not possible, the foods should be mixed for no more than 2-day intervals.

As many of the vitamins are water-soluble, leaching in the water is an important avenue of loss of vitamins from the diet. Diets should be prepared with as little water as possible, and the ingredients should be bound by standard methods (salt and spleen). Preparation of foods with blower and cement or dough mixer reduces leaching considerably, and use of

the new pelleted types of foods practically eliminates the loss of ingredients when the food is placed in water.

SUMMARY

1. Vitamins are catalytic substances required by all forms of life.

2. No clear-cut need has been established for the fat-soluble vitamins D, E, K, and A in trout.

3. It has been established that trout require 10 of the water-soluble vitamins (thiamine, biotin, pantothenic acid, riboflavin, pyridoxine, folic acid, B_{12}, niacin, inositol, and choline) in the diet.

4. All of the required vitamins must be present in all trout diets to assure the prevention of vitamin deficiencies.

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In the summer of 1956 fish were harvested from waters of the U. S. Fish-Cultural Station at Guttenberg, Iowa, during a barrage of flashbulbs and shotguns. On one occasion, 72 egrets and 11 blue herons were counted over a single 10-acre pond.

When men reported for spawning operations at a salmon trap near the U. S. Fish-Cultural Station near Quilcene, Washington, one morning in December 1956, they found that work at that trap had already been done. Missing from the net rack was a cotton seine used to fish the trap; missing from the trap were three to four hundred chum salmon that had been there the night before.

AN INVESTIGATION OF CARBON DIOXIDE, AMMONIA, AND HYDROGEN SULFIDE AS FACTORS CONTRIBUTING TO FISH KILLS IN ICE-COVERED LAKES

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MANY FERTILE, SHALLOW LAKES OF SOUTHERN MINNESOTA often lose some or all of their fish through winterkill under the ice. It is necessary to investigate winter conditions in these lakes periodically to judge whether a winterkill is apt to occur. If a kill appears likely, game fish are removed by seining under the ice or the lake is opened to "promiscuous fishing" and the usual fishing restrictions are lifted. These management steps have been based for a number of years on dissolved-oxygen concentrations and the history of the waters. Sometimes, however, there are anomalous situations where some fish survive in a lake very low in oxygen or where kills occur at apparently safe levels.

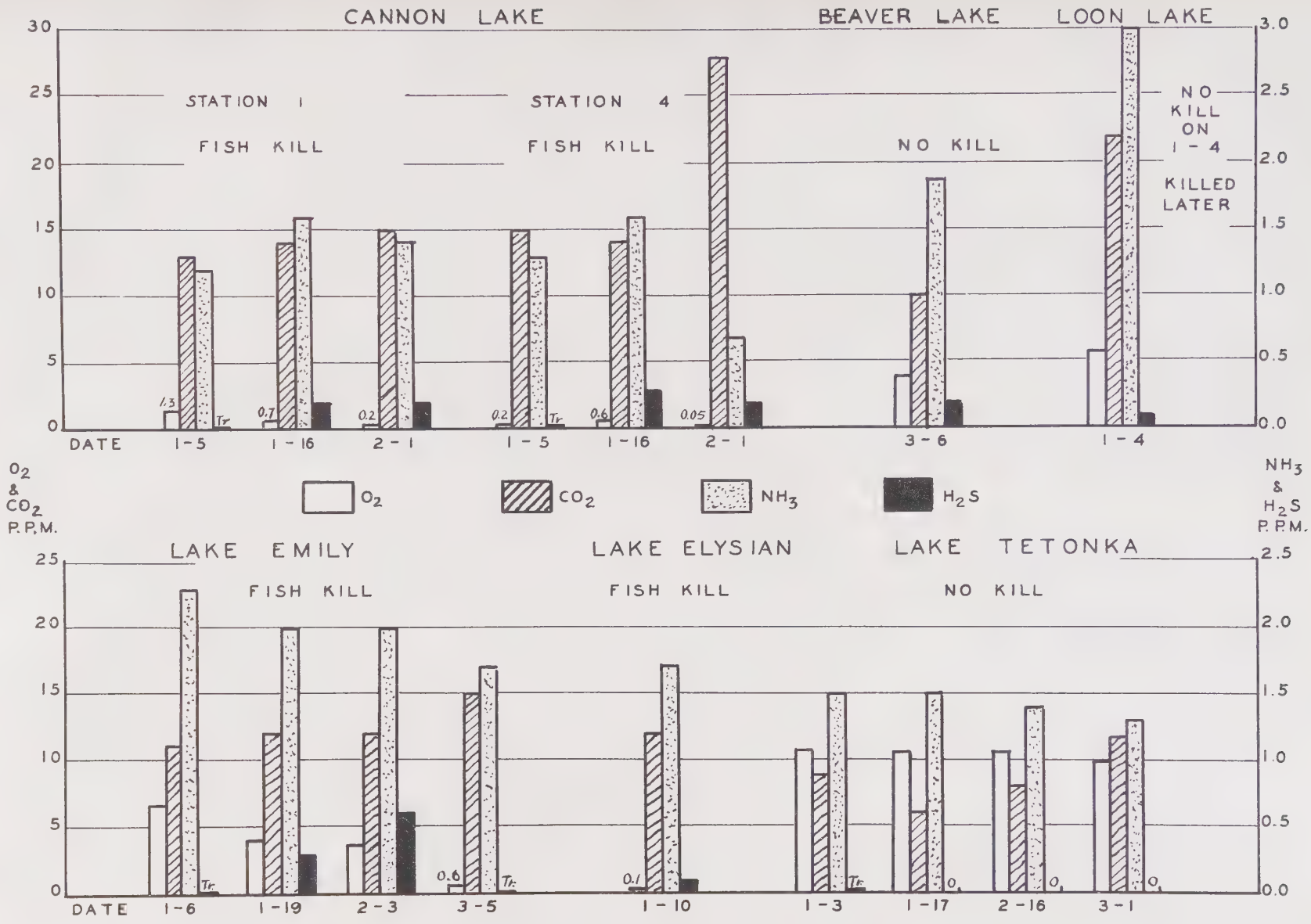
It has long been known that concentrations of dissolved oxygen vary somewhat throughout a lake at any depth, and that bottom waters are depleted of oxygen first. Also, it has been demonstrated that fish can be conditioned to low oxygen (Moore 1942, and others), and that decrease in oxygen may be associated with increase in carbon dioxide and other gaseous products of decay. For a review of the literature, the reader is referred to Greenbank (1945) and Cooper and Washburn (1949).

In the winter of 1955-56, the present study was made on six southern Minnesota lakes to learn more about the relationships between levels of dissolved oxygen, carbon dioxide, hydrogen sulfide, ammonia, and winterkills of fish. The six lakes selected for study are in the farming country of south-central Minnesota. They are: (1) Elysian, 2,700 acres with a maximum depth of 10 feet; (2) Cannon, 1,470 acres with a maximum depth of 10 feet; (3) Loon, 172 acres with a maximum depth of 10 feet; (4) Emily, 127 acres with a maximum depth of 35 feet; (5) Tetonka, 1,209 acres with a maximum depth of 35 feet; and (6) Beaver, 107 acres with a maximum depth of 31 feet. These lakes have sandy or gravelly shorelines but silty bottoms. Loon and Emily support good stands of aquatic vegetation, but the others are mostly bare of weeds because of carp and heavy plankton growth.

During the period of investigation (the first 3 months of 1956), Cannon, Emily, Loon, and Elysian winterkilled; Tetonka and Beaver did not.

Methods

Water samples were taken through the ice on these six lakes on the dates shown on the chart, as well as on some intermediate dates. A Kemmerer water sampler was used. Dissolved oxygen samples were "fixed" in the field by the addition of reagents and analyzed by the unmodified Winkler method. Water samples for other analyses were collected in chemically clean bottles and analyzed at the laboratory at the Waterville Fisheries Headquarters within 2 hours of collection. Samples were held at collection



Dissolved gas relationships in ice-covered Minnesota lakes, showing conditions associated with winterkill and survival of fish. (Drawing courtesy of the author)

temperature and protected from shaking and jarring.

Methods described by Ellis, Westfall, and Ellis (1948) were used for ammonia and carbon dioxide. Ammonia was determined by ocular comparison of samples with freshly prepared standards. Hydrogen sulfide was determined by the titration method described by the American Public Health Association (1936), using freshly prepared standard iodine solutions.

Samples were usually taken at several depths, but in this paper only samples taken 2 feet under the ice are considered. The conditions in this upper stratum are critical to fish.

Discussion

Some of the more pertinent data are shown on the chart. It will be observed that all the fish kills observed were associated with low oxygen levels--0.6 p.p.m. or less. Distressed or dead fish were found in Cannon Lake when oxygen levels declined from 1.6 to 0.4 p.p.m., and in Lake Emily when oxygen declined from 1.4 to 0.2 p.p.m. During these oxygen declines there was no appreciable increase in the concentrations of the other dissolved gases. In Lake Elysian, where the oxygen was low and a kill had begun prior to the investigation, levels of the other gases were not much higher than in Beaver Lake or in Lake Tetonka, where no kill occurred. At Loon Lake, high concentrations of ammonia (3.0 p.p.m.) and carbon dioxide (22.0 p.p.m.) were noted on January 4, at which time dissolved oxygen stood at 5.6 p.p.m. and fish in lively condition were being taken in rescue operations. The ammonia level was higher than 2.5 p.p.m., which was considered by Ellis, Westfall, and Ellis (1948) as being generally detrimental or lethal.

From two lakes--Tetonka, which had no winterkill, and Emily, which killed--a series of samples were taken from three depths at the same stations and analyzed. Depths of samples were 1, 15, and 30 feet in Lake Emily where the water depth was 35 feet, and 2, 6, and 15 feet in Lake Tetonka where the water depth was 17 feet. As is well known, dissolved oxygen declined with depth. The spread between dissolved oxygen contents of top and bottom waters in Lake Emily decreased as the season progressed, being 8.1 to 0.0 p.p.m. on January 6, 3.5 to 0.2 on February 3, and 0.2 to 0.6 on March 5. The decline of oxygen in the lower waters was less in Lake Tetonka, the spread from top to bottom being 11.0 to 11.0 p.p.m. on January 3, 9.8 to 9.0 on February 2, and 10.0 to 5.8 on March 1.

Carbon dioxide content showed no consistent difference with depth throughout the winter, the spread from top to bottom in Lake Emily being 9.0 to 11.0 p.p.m. on January 6 and 17.0 to 10.0 on February 17. In Lake Tetonka the spread was 9.0 to 9.0 p.p.m. on January 3, 12.0 to 11.0 on February 2, and 12.0 to 10.0 on March 1. The ammonia concentrations in Lake Emily were somewhat greater in the deeper water, the spread from top to bottom being 2.1 to 3.0 p.p.m. on January 6 and 1.7 to 2.5 on February 17. In Lake Tetonka, however, ammonia concentrations differed little from top to bottom, the spread being 1.5 to 1.5 p.p.m. on January 3, 1.5 to 1.5 on February 2, and 1.3 to 1.2 on March 3. Hydrogen sulfide showed little difference with depth in Lake Tetonka, the spread from top to bottom being trace to 0.0 p.p.m. on January 3 and 0.0 on both top and bottom waters at four subsequent sampling dates (the last of which was March 1). Similarly, in Lake Emily the spread from top to bottom was trace to 0.3 p.p.m. on January 6, 0.4 to 0.6 on February 3, and trace to 0.4 on March 5.

It is concluded that the dissolved oxygen level is the best basis for forecasting winterkills. Although high concentrations of ammonia, carbon dioxide, and hydrogen sulfide may be associated with winterkills, oxygen appears to be the most causative factor. As ammonia, carbon dioxide, and hydrogen sulfide are associated with decomposition and thus with conditions of reduction and low oxygen, it is likely that winterkills are usually related to concentrations of these gases as concurrent

phenomena rather than as the cause and effect. There is the possibility, demonstrated experimentally by Black, Fry, and Black (1954), that increasing concentrations of carbon dioxide reduce the ability of the fish to utilize oxygen; but the present evidence indicates that it is not possible to separate the individual effects of the four gases considered. Dissolved oxygen apparently provides the best single index of suitability of the water for fish in lakes where winterkill is a potential.

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SILVERSIDES PLANTED IN URUGUAY

Planting more than 1 hundred thousand Pejerreyes, or silversides (Odonthestes bonaerensis), in the Canelon Grande Dam in Uruguay last February marked the start of a campaign to stock all suitable waters with fish. The project was undertaken by the Servicio Oceanografico y de Pesca, the Consejo Departamental de Canelones, and the Departamento de Investigaciones Pesqueras y Biologia Marina, Facultad de Veterinaria, Universidad de la Republica del Montevideo, Uruguay.

Dr. Victor H. Bertullo, chief of the Departamento de Investigaciones Pesqueras y Biologia Marina, reports that the eggs were flown in from Argentina and incubated near the Canelon Grande Dam. While held in a 10,000-gallon Australian tank that provided some natural food, the young fish were given fresh bovine blood and mashed liver. When about 2.5 months old and 2 to 2.5 inches long, the fish were planted in the best locations in the dam.

"This is the first step in the plan to repopulate the dam," writes Dr. Bertullo. "In the next 5 years we shall plant about 1 million Pejerreyes each year, taking into consideration the fact that the dam has a surface of 1,000 hectares.

"In our Faculty, the University of Montevideo will build a pond (300 by 120 feet, with an average depth of 10 feet) for a study of the spawning and planting of silversides throughout the country. Our Department now has a section devoted to fish culture and will study several other excellent species."

A PRELIMINARY NOTE ON THE USE OF DI-N-BUTYL TIN OXIDE TO REMOVE TAPEWORMS FROM FISH

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A NUMBER OF DRUGS GIVEN ORALLY have been used to remove tapeworms from the intestinal tract of fish. Of these drugs, kamala has been the most commonly used.

Although used for many years as a taeniacide for chickens and other domestic animals, kamala is now in disfavor as an anthelmintic because of many objectionable features. When kamala was used, it was necessary to starve the host animal for a prescribed period before and after treatment. Jones (1954) concluded that starving infected chickens produced about the same results as the administration of kamala. Reid (1940) determined that starving infected chickens 20 to 48 hours caused segments of the tapeworms to be passed. He reported (1942) that the scolex was not affected by starving, and that infective segments appeared in the feces when the infected chickens were fed regularly after the starvation period.

As kamala is definitely in disfavor as a taeniacide, it was decided that some of the newer anthelmintics might be more effective in removing tapeworms from fish. Edgar (1956) found that several tin compounds were effective in removing certain species of tapeworms from chickens. Accordingly, one of these compounds, di-n-butyl tin oxide, was tested to determine its effectiveness in removing tapeworms and intestinal flukes from fish.

Materials and Methods

Approximately 1,000 channel catfish, Ictalurus lacustris punctatus (Rafinesque), were obtained from a commercial hatchery in Arkansas. A number of these fish died in transit and were examined for internal parasites. Approximately 75 percent of those examined were infected with a tapeworm, Corollobothrium fimbriatum Essex, and with a fluke, Alloglossidium corti. Individuals from this shipment were used in testing the anthelmintic properties of di-n-butyl tin oxide.

As the pre-treatment level of infection could not be determined, 150 fish were taken at random from the population, divided into 6 groups of 25 each, and placed in separate feeding tanks. Five fish were taken at random from each tank and autopsied to determine the approximate level of infection prior to treatment.

The test animals, which had an average weight of 30 grams per fish, were fed a commercial meat-and-vegetable diet at the rate of 3 percent of the body weight per day. Two groups of test animals each received a total of 250 milligrams of di-n-butyl tin oxide per kilogram of body weight of fish. This medication was administered by incorporating 0.28 percent di-n-butyl tin oxide into the commercial food and feeding this mixture at the rate of 3 percent of their body weight per day. Kamala was used at the rate recommended by McKernan (1940). It was mixed into the food at the rate of 2 percent by weight, and the mixture was fed daily to the fish at the rate of 3 percent of their body weight. Two groups of fish received the unmedicated commercial diet. After the 3-day treatment, all of the test animals were

TABLE 1.--The efficacy of di-n-butyl tin oxide and kamala in removing Corollobothrium fimbriatum from channel catfish

Treatment	Number of test fish per treatment	Replication 1			Replication 2			Totals for 1 and 2			Efficacy
		Number of infected fish	Number of worms	Average number of worms per fish	Number of infected fish	Number of worms	Average number of worms per fish	Number of infected fish	Number of worms	Average number of worms per fish	
Untreated control--	20	5	17	3.40	4	13	3.25	9	30	3.33	------
Kamala-----	20	2	5	2.50	3	7	2.30	5	12	2.40	27.90
Di-n-butyl tin oxide	20	0	0	.00	0	0	.00	0	0	.00	100.00

TABLE 2.--The efficacy of di-n-butyl tin oxide and kamala in removing Alloglossidium corti from channel catfish

Treatment	Number of test fish per treatment	Replication 1			Replication 2			Totals for 1 and 2			Efficacy
		Number of infected fish	Number of worms	Average number of worms per fish	Number of infected fish	Number of worms	Average number of worms per fish	Number of infected fish	Number of worms	Average number of worms per fish	
Untreated control--	20	6	10	1.40	4	12	3.00	10	22	2.20	-------
Kamala-----	20	2	3	1.50	4	8	2.00	6	11	1.10	50.00
Di-n-butyl tin oxide	20	0	0	.00	0	0	.00	0	0	.00	100.00

held in the feeding tanks for 1 week and each group received the commercial diet at the rate of 3 percent of the body weight per day. At the end of this feeding period, all of the test animals were autopsied to determine the efficacy of the materials tested. The calculated efficacies reported in this paper are based on the "controlled test" described by Moskey and Harwood (1941).

Attempts were made to determine the toxicity of di-n-butyl tin oxide and di-n-butyl tin dilaurate to largemouth bass, Micropterus salmoides. Di-n-butyl tin oxide was given orally by capsule, and di-n-butyl tin dilaurate was injected into the body cavity of a minnow which was then fed to the bass. The dosage rates were 150, 250, 350, and 500 milligrams per kilogram of body weight of the bass. Four replications of each dosage rate were used.

Results and Discussions

Of the 30 test animals autopsied prior to treatment, 33 percent were infected with Corollobothrium fimbriatum and 26 percent were infected with Alloglossidium corti. The average number of tapeworms and flukes per fish was 2.50 and 3.25, respectively. This was considered the base level of infection prior to treatment.

The results of the medication with di-n-butyl tin oxide are presented in tables 1 and 2. Although the percentage of infection and the average number of worms per fish were low in the unmedicated controls, it was significant that in both replications 100 percent efficacy was indicated for di-n-butyl tin oxide against both the tapeworm and the fluke. For kamala, efficacies of 27.9 and 50.0 percent were indicated for tapeworm and fluke, respectively. These data seem to justify the inference that kamala is not an efficient anthelmintic.

Tests of the toxicity of di-n-butyl tin oxide and di-n-butyl tin dilaurate to largemouth bass were inconclusive. No toxic symptoms developed in test animals fed at the rates of 150 and 250 milligrams per kilogram of body weight of bass. In all replications where 350 and 500 milligrams per kilogram of body weight were used, both in the capsules and in the minnows, the test fish regurgitated the material.

Summary

These data indicate that di-n-butyl tin oxide has anthelmintic properties against the fish tapeworm, Corollobothrium, and the intestinal fluke, Alloglossidium. When administered at the rate of 250 milligrams per kilogram of body weight, the chemical removed 100 percent of these parasites.

SODIUM SECONAL AS A SEDATIVE FOR FISH

To hold to a minimum the external injuries to fish in transit and to increase with impunity the usual or maximum fish-to-water ratio, fishery workers have been seeking a safe means of reducing the rate of metabolism of fish. Francis H. Bezdek, biologist working for the Soil Conservation Service at Lisbon, Ohio, has recently reported on a trial of sodium seconal as a sedative for fish.

Forty-five adult (2.5- to 3-inch) minnows (Pimephales promelas) were placed in 1.5 quarts of water at a temperature of 50° F. A 0.75-grain (0.05-gram) capsule of sodium seconal was added to the water.

The effects of the drug began to show about half an hour after its administration. The symptoms among the fish included sluggishness, rolling from side to side, and retarded breathing.

Ten fish were removed to fresh water after 1 hour in the drugged solution. They revived in about 3 hours. The 35 other fish were put in fresh water after spending 3 hours in the drugged solution. All these fish were completely revived in 5 hours.

EFFECT OF *RAUWOLFIA* ALKALOIDS ON THE OXYGEN CONSUMPTION RATE OF HATCHERY-REARED RAINBOW TROUT

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THE ROOT OF THE PLANT Rauwolfia serpentina Bentham, a shrub indigenous to India, has been employed for centuries in native medicine to relieve various central nervous derangements, both psychic and motor, including anxiety, excitement, maniacal behavior, and epilepsy. More recently it has been tried with beneficial results on human hypertension, first by Indian workers and subsequently by others in this country. In addition to the drug's effect in lowering blood pressure in hypertension, patients seem to feel relaxed and free of preexisting anxiety and tension when under its influence (Plummer et al. 1954). Unlike barbiturates, it renders patients less active without inducing sleep. This action, peculiar to reserpine and similar alkaloids of R. serpentina, has been called a tranquilizing effect. There is evidence that this state of quiescence is distinctly different from sedation induced by the barbiturates (Rinaldi and Himwich 1955). An excellent bibliography in this field is presented by Kline (1954).

The hypothesis has been advanced that postplanting delayed mortality of hatchery-reared rainbow trout, Salmo gairdneri Richardson, may be due to nervous or physical shock induced by the ordeal of transportation (Horton 1956). In an effort to reduce excessive excitement, these fish have been subjected to various concentrations of sodium amytal and methyl pentynol during transport without significantly lowering losses of this nature.[1/] Upon perusal of reports of the ability of reserpine (Serpasil) to arrest hypertension in warm-blooded animals (Plummer et al. 1954) and to act as a sedative on Siamese fighting fish, Betta splendens (Turner and Carl 1955), it was decided to determine whether the drugs might have metabolism-reducing effect on hatchery trout.

Methods

The total metabolic activity of an animal may be roughly measured by its rate of oxygen consumption (cubic centimeters per gram per hour). Comparative tests were run to demonstrate any difference in oxygen uptake between animals subjected to reserpine, rauserfia, or raunormine and animals in a control aquarium. Rainbow trout were collected from the Roaring River Trout Hatchery at Scio, Oregon, and held in aerated tap water which had been passed through an activated charcoal filter.

Tests were conducted in three liter jars placed in a circulating-water bath maintained at 15° C. Into each jar were placed 2.5 liters of water from the holding aquaria. Aerators, test animals (15 fry averaging 0.22 gram each), and chemical were added immediately and left for an acclimatization period. After 30 minutes, the aerators were removed and 0.25 inch of white mineral oil was poured into each jar to prevent inward diffusion of oxygen at the surface of the water (Amberson et

[1/] Horton, H. F. Unpublished material.

al. 1924). A thermometer and sampling tube mounted in a vented stopper were placed in each of the test containers. A water sample was withdrawn immediately and every 20 minutes thereafter until 140 minutes had elapsed.

Dissolved oxygen determinations were made according to the procedure outlined in Tully (1950), which follows the basic Winkler method and is accurate to about 0.02 cubic centimeter per liter. The oxygen consumption rate was calculated for each 20-minute interval. The 7 collective figures for each aquarium were then treated as a statistical sample and analyzed by use of a t-test at the 95 percent confidence level.

Reserpine, rauserfia, and raunormine were tested at concentrations of 10, 100, and 1,000 micrograms percent. It has been reported that Siamese fighting fish exhibited symptoms of sedation when subjected to 12 micrograms percent reserpine (Turner and Carl 1955).

Results and Discussion

In all tests (including controls), there was a very minor reduction in consumption rate as time progressed and the available oxygen supply decreased. There was not, however, any significant difference in consumption between control and experimental groups, regardless of concentration or chemical employed (see table). In this regard, Hafkenschiel, Sellers, King, and Thorner (1955) determined that cerebral oxygen uptake in man remained unchanged 1 hour after intravenous injections (40 to 108 micrograms per kilogram) of reserpine.

At no time did the test animals exhibit symptoms of sedation or tranquilization. Test fish were observed to compete immediately for food in a normal manner when placed in company with nonexperimental fish.

There was a noticeable dilation of the melanophores in the test animals. This effect was barely visible at 10 micrograms percent of all drugs and increased in intensity with stronger concentrations. At 1,000 micrograms percent, the test fish were very black, in contrast to the fish in the control aquaria. The first color changes began approximately 30 minutes after exposure and persisted for 1 to 3 days, depending upon dosage received. Similar color changes in the Siamese fighting fish were observed by Turner and Carl (1955) while working with reserpine, and by Abramson and Evans (1954) while working with lysergic acid diethylamide (LSD 25), an antagonist of reserpine.

MEAN OXYGEN CONSUMPTION VALUES IN TESTS WITH THREE RAUWOLFIA ALKALOIDS, April 12 to May 23, 1956

Chemical	Microgram percent	Oxygen consumption	
		Test	Control
		(cc./gm./hr.)	
Reserpine	10	0.41	0.37
	100	.40	.37
	1,000	.29	.37
Raunormine	10	.34	.39
	100	.35	.39
	1,000	.37	.39
Rauserfia	10	.29	.34
	100	.33	.34
	1,000	.31	.34
Pooled mean----------		.34	.37

Conclusions

1. Reserpine, rauserfia, and raunormine did not significantly lower dissolved oxygen consumption of hatchery-reared rainbow trout under the concentrations and conditions tested.

2. A noticeable darkening of the fish exposed to these Rauwolfia alkaloids was observed. This phenomenon increased with stronger chemical concentrations.

3. No tranquilizing effect or sedation was observed among animals subjected to various concentrations of these chemicals.

Acknowledgment

Drugs were made available, courtesy of S. B. Penick and Company, through Messrs. Earl Goss and George Motoasca.

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STUDY OF TISSUE IN PANCREATIC NECROSIS

Methods of tissue culture have been applied to the study of infectious pancreatic necrosis in the Microbiological Laboratory at the U. S. Fish and Wildlife Service's Leetown Station, Kearneysville, West Virginia.

In these preliminary trials a saline extract was made of tissues from brook trout fry that had the "whirling" symptoms of pancreatic necrosis. This extract was clarified, filtered to remove bacteria, and then used to inoculate growing cultures of trout tissue. Within 4 days the inoculated cultures degenerated, while uninoculated control cultures did not exhibit such a change. Similar changes were observed, however, when small amounts of fluid from degenerated cultures were added to other growing cultures. The calculated dilution of the original saline extract was 1,600-fold.

These results may be cautiously interpreted as evidence supporting a virus cause, but at this stage of the study they are not proof that pancreatic necrosis is due to a virus infection.

CARRYING RACK FOR OUTBOARD BOATS AND MOTORS

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HAULING SMALL BOATS AND OUTBOARD MOTORS over many miles of rough road is usually difficult and inconvenient. At the best this equipment usually receives more punishment while being transported than while being used. Trailers often provide a good method of transportation for the boat but seldom help in transporting motors. Trailers also can become a nuisance on back-country roads or in traffic. Pickup trucks that are equipped with the combination unit (figure 1) can transport boats and motors over almost any forest trail, can be handled easily in traffic, and can be put into most parking spaces.

The unit is of simple but strong construction and has proved itself practical during three seasons of use. It has been used to carry a 12-foot boat and one or more outboard motors. This unit can be constructed for any pickup truck that is equipped with stake pockets.

The dimensions of the boat rack are not shown on the drawing because they depend on the size of the pickup bed and the boat to be used. It was found that the boat was at a convenient height when the bottoms of the notches in the front support were 40 inches above the pickup bed and the bottoms of the notches in the rear support were 37 inches above the bed.

The crosspiece in rear mount type A (figure 2) is handy when a large load of equipment is being tied down, or when one person is loading a boat. The rear mount type B (figure 3) is handy in that it leaves the entire space under the boat open and facilitates the removal of bulky equipment without taking down the boat rack.

FIGURE 1.--Truck equipped with the combination unit.

(Illustrations courtesy of the authors)

The tie-down ropes for this type of boat rack should always be attached to the pickup bed, not to the uprights on the rack, as this will prevent the loss of the boat should the uprights bounce out of the stake pockets.

All lumber used in the rack should be finished.

Following is an itemized list of the materials:

Front support:

2	oak uprights ----	1-1/2"	x 2-1/4"
1	crosspiece ------	1"	x 6"
1	crosspiece ------	1"	x 12"
2	cleats ----------	1"	x 6"
4	machine bolts ---	7/16"	x 5"
4	machine bolts ---	7/16"	x 4"
16	flat washers ----	7/16"	
8	wood screws -----	No. 8	x 1-1/4"

56 inches of 1-inch webbing, and shingle nails to affix it.

FIGURE 2.--Rear of boat rack, type A.

FIGURE 3.--Motor mount and rear of boat rack, type B.

Rear mount, type A:

2 oak uprights ----	1-1/2"	x 2-1/4"
1 crosspiece ------	2"	x 6"
2 strap iron cleats, 5-1/2" long ---	1/8"	x 1"
4 machine bolts ---	7/16"	x 5"

40 inches of 1-inch webbing, and shingle nails to affix it.

Rear mount, type B:

2 oak uprights ----	1-1/2"	x 2-1/4"
2 strap iron, 10-1/2" long, to make hangers	1-1/2"	x 1/4"
4 machine bolts ---	7/16"	x 2-1/2"
2 machine bolts ---	7/16"	x 3"
12 flat washers ----	7/16"	
6 lock washers ----	7/16"	

15 inches of 1-1/4-inch radiator hose to cover strap-iron hangers.

These mounts were constructed and placed in the stake pockets of the pickup on which they were to be used. The boat was turned upside down, stern forward, and placed on the mounts in the position in which it was to be carried. The outline of the boat was traced on the mounts. This outline plus enough more wood to allow for 2 layers of webbing was notched out approximately 2 inches deep. With the type-B rear mount, no crosspiece was used. The hangers on which the boat rests (figure 3) were formed from strap iron (1/4 by 1-1/2 inches) and padded with radiator hose.

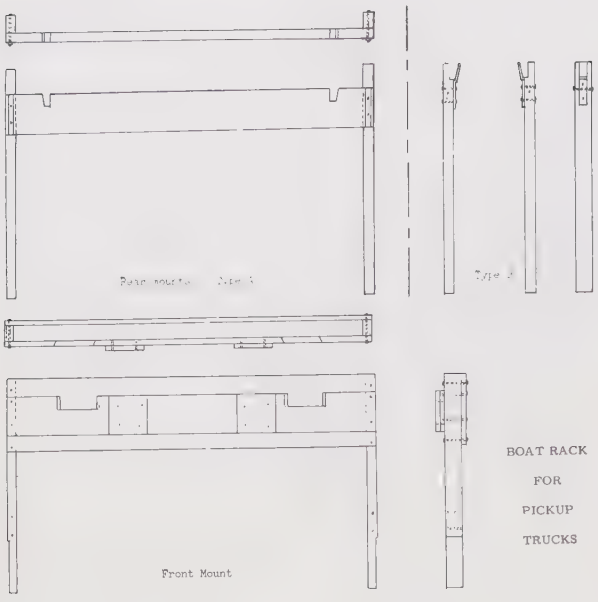
The dimensions for the motor mount are determined by the vehicle on which the rack is to be mounted, by the make, model, and size of motors to be transported, and by the boat to be transported. Dimensions given here are for Johnson Motors, 1954 and 1955 models, up to 10 horsepower, carried under a 12-foot Aero-Craft boat.

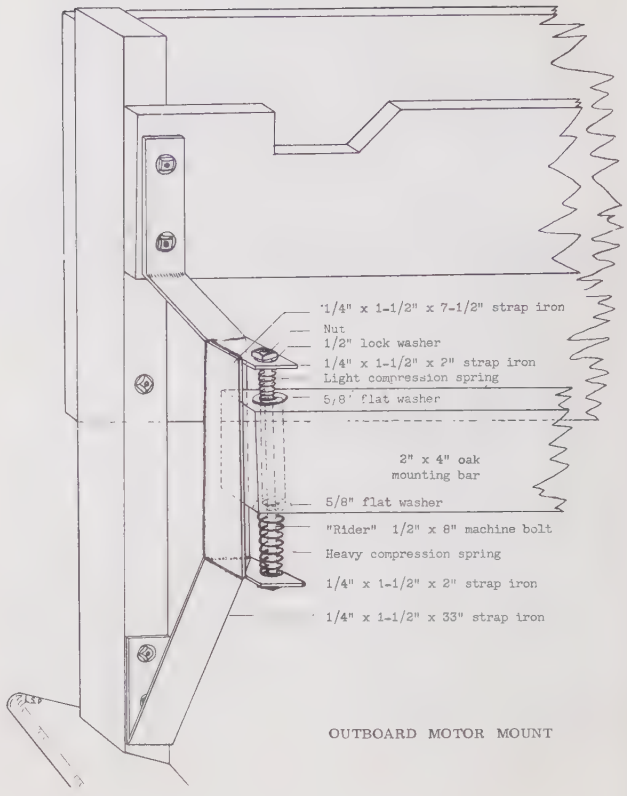
The following materials are required for construction of the motor mount:

1 oak mounting bar	2"	x 4"
2 strap iron, 7-1/2" long ----	1-1/4"	x 1-1/2"
2 strap iron, 33" long--------	1/4"	x 1-1/2"
4 strap iron, 2" long --------	1/4"	x 1-1/2"
2 heavy coil springs		
2 light coil springs		
2 machine bolts ----	1/2"	x 8"
4 flat washers -----	5/8"	
2 lock washers -----	1/2"	

The two 33-inch pieces of strap iron are bent identically and must give at least 6 inches of clearance for the "forehead" of the motor. The top of the mounting bar must be at least 31 inches from the floor of the truck bed, and there must be at least 18 inches between the top of the mounting bar and the boat (boat seat in the Aero-Craft) above the head of the motor.

The two 33-inch straps have a winged-V shape when completed, the vertex of the V flattened for 7-1/2 inches (see drawing).




1/4" x 1-1/2" x 7-1/2" strap iron
Nut
1/2" lock washer
1/4" x 1-1/2" x 2" strap iron
Light compression spring
5/8" flat washer
2" x 4" oak
mounting bar
5/8" flat washer
"Rider" 1/2" x 8" machine bolt
Heavy compression spring
1/4" x 1-1/2" x 2" strap iron
1/4" x 1-1/2" x 33" strap iron
OUTBOARD MOTOR MOUNT

The two wings of the strap iron are fastened to the boat rack uprights with two bolts at each end. When completed, there will be a right- and a left-hand bracket (figure 3).

Each of the two 7-1/2-inch straps is welded along the 7-1/2-inch flattened vertex of a bracket. At each end of this welded strap will be one of the four 2-inch pieces of strap iron welded in the angle formed by the 33- and 7-1/2-inch pieces. A 1/2-inch hole is drilled in each of the short straps to take the "rider" bolt that is put through each end of the mounting bar.

The mounting bar is cut to length so that plenty of end play is allowed. A slotted hole (5/8 by 1 inch) is drilled edgewise through each end of the bar. This allows play to keep the bar from binding when loaded. The compression springs under the bar should be of such strength as to give slightly with the weight of the lightest motor on the mounting bar. The compression springs above the bar merely eliminate any rebound as the lower springs are compressed by a sudden jolt or shock. A flat washer is placed on each side of the bar to keep the springs from wearing into the wood.

The placing of the two rider bolts completes the assembly of the mount. Each bolt is passed through the bottom of the bracket, the heavy compression spring, a flat washer, the mounting bar, another flat washer, the light compression spring, the other side of the bracket, and finally through a lock-washer; thereafter the nut is tightened down (see drawing).

Care should be taken to see that the mounting bar rides free on the rider bolt and does not bind on any bracket. A little grease in the hole at each end of the bar will help to secure a smoother ride.

Time required to construct the combination rack and motor mount is about 6 hours after materials are assembled.

Lake Superior is the largest body of fresh water on the globe.

A STOMACH SAMPLER FOR LIVE FISH

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CRITICAL STUDIES OF THE FOOD HABITS OF GAME FISH are often hampered by reluctance or inability of the investigator to obtain food from an adequate number of fish stomachs. This is especially true in waters having intensive sport fisheries or where the species under investigation is in short supply. If, to obtain stomachs, the investigator kills a large number of fish, he may damage public relations or substantially reduce the numbers of a species already scarce.

To circumvent these difficulties, Dubets (1954) designed an elongate metal funnel that, when inserted into the fish's esophagus, enabled the worker to view the stomach contents without injury to the fish. This method, though adequate for gross examination of stomach contents of larger fish, is not satisfactory for detailed analysis of the entire stomach contents. The device described in this paper permits the removal of the entire stomach contents from fish of all sizes without injury to the fish. It is an improvement over the method described by White (1930) for extracting food from the stomachs of live trout. He inserted a glass tube into the gullet and, by pressing the side of the fish, forced food from the stomach into the tube. The present device uses paired copper tubes and a "flusher" to remove material from the stomach with water.

Description

Two copper tubes, one smaller than the other, are soldered together and shaped at one end for easy access to the esophagus (figure 1). The opposite end of the larger tube is flared and fitted into a hole in the cover of a standard specimen bottle (figure 2). Water is pumped through the smaller tube into the stomach of the fish by means of a rubber suction bulb with a check valve at each end to produce a unidirectional flow (figure 2). The stomach contents are washed through the larger tube into the attached specimen bottle. Here they may be preserved and labeled for future reference. A fine-mesh copper screen soldered over another hole in the bottle cap allows excess water to escape. Models of the sampler have been constructed with tubes of various diameters to accommodate fish 4 to 15 inches in length. Table 1 shows the dimensions of the copper tubing used for the outlet and inlet lines on the various samplers.

Operation

The fish is held with its head downward. The tube of the sampler is placed in the open mouth (figure 2) and then inserted slowly into the esophagus. The rubber bulb (which is connected by a short piece of rubber tubing to a supply of water) is squeezed, and water is forced through the inlet tube into the stomach. (If the tip of the sampler is not completely enclosed by the esophagus, a spray of water will be released through the gills.) Flushing water and stomach contents pass through the larger tube and into the attached specimen jar. Usually about 150 cubic centimeters of water will be sufficient for adequate flushing, but the amount of water needed depends on the size of the fish and the nature of the stomach contents. Large

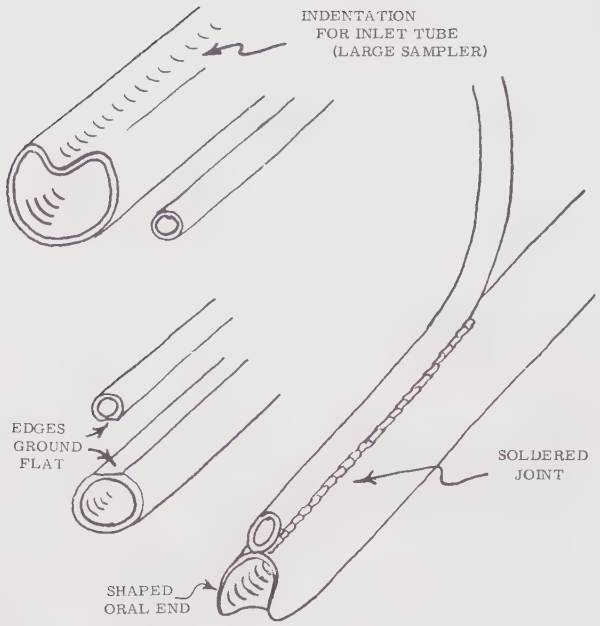

FIGURE 1.--Joining the inlet and outlet tubes for the oral or upper end of the sampler.

(Drawings courtesy of the author)

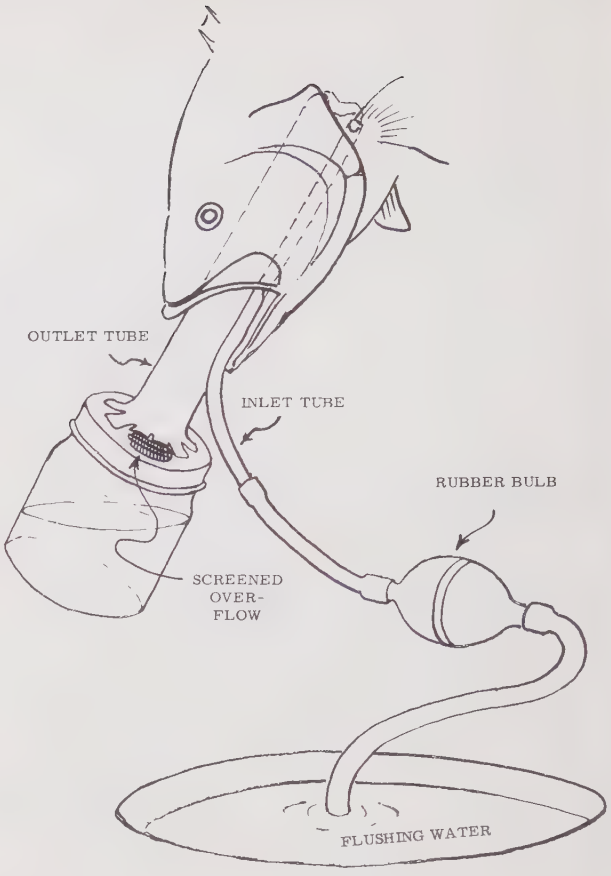

FIGURE 2.--Complete assembly of the stomach sampler. Rubber bulb is of the type used on syringes.

fish and large undigested food particles require most flushing. It is necessary at times to use forceps to extract from the stomach food particles too large to pass through the outlet tube. The screened hole in the bottle cover permits escape of excess flushing water without loss of food.

The size of sampler to use is determined by experience. Table 2 lists the maximum and minimum sizes of fish of several species on which samplers of the various sizes have been used successfully. Large undigested particles in the stomach can be detected by feeling the side of the fish; when such particles are present, the largest sampler that can be accommodated should be used. Smaller samplers can be used effectively when the stomach contains only small foods.

Preliminary observations indicate that mortality associated with the use of this apparatus is negligible. Forty perch, 20 of which had been subjected to the stomach sampler, were held for 24 hours after treatment. At the end of this period no mortality was observed in the treated lot, but 5 fish of a control lot died. In a study lake where this sampler was in use, all the treated fish were fin-clipped.

TABLE 1.--Size of copper tubing for stomach sampler

[Dimensions in inches]

Sampler No.	Inlet tube			Outlet tube		
	Diameter		Length	Diameter		Length
	Outside	Inside		Outside	Inside	
1 ----	1/8	1/16	5	1/4	3/16	5
2 ----	1/8	1/16	6	5/16	7/32	6
3 ----	3/16	3/32	7	1/2	13/32	7
4 ----	1/4	3/16	8	5/8	9/16	8
5 ----	1/4	3/16	9	3/4	5/8	9

TABLE 2.--Minimum and maximum lengths of fish suitable for several sizes of samplers

Species	Range in length of fish sampled with Sampler No.:				
	1	2	3	4	5
Northern pike---inches--	---------	---------	10.0 - 17.9	12.3 - 15.5	15.6 - 21.1
Black crappie-----do.---	---------	4.5 - 7.8	7.1 - 12.3	----------	----------
Bluegill----------do.---	4.2 - 5.1	5.0 - 8.9	----------	----------	----------
Yellow perch------do.---	---------	4.6 - 7.3	6.6 - 9.5	----------	----------
Walleye pike------do.---	---------	6.3 - 7.5	7.7 - 14.3	----------	15.3 - 17.8
Largemouth bass---do.---	---------	3.7 - 5.7	6.0 - 12.6	----------	10.4 - 15.0

To date, no mortality has been observed and a substantial number of marked fish have been recovered.

The efficiency of the sampler was checked by post-mortem examination of a number of stomachs from fish that had been subjected to the sampler. Of 25 perch, 15 walleye pike, and 5 smallmouth bass stomachs that were examined, food remains were found in only a single stomach--that of a perch which had retained the remains of 1 small fish. All other stomachs were completely empty after being flushed with the sampler.

Construction Details

The ends of the tubes must be shaped as shown in figure 1 to permit access to the stomach without injury to the wall of the esophagus. Edges should be rounded. On the larger samplers (Nos. 4 and 5 in table 1), it is necessary to join the two supply tubes as shown in figure 1 so that the general rounded shape of the apparatus may be preserved. A hardwood dowel about the diameter of the inlet tube may be used to shape a depression into which the smaller tube is placed. The depression is more easily formed if the tube wall is thinned by grinding before it is hammered into shape. After the depression has been formed, the tubes are soldered together for the lower third of the length of the outlet tube. The inlet tube is bent to one side at the other end to receive the rubber tubing from the flushing bulb.

To make smaller samplers (Nos. 1, 2, and 3 in table 1), it is necessary to grind the opposing surfaces of the tubes flat before soldering them together.

The end of the outlet tube that is connected to the bottle top is slit longitudinally several times for about one-half inch to form flaps. These are bent outward and soldered to the outside of the bottle top. Two holes are cut in the bottle cap--the larger hole for the tube and the other hole for the overflow. A piece of fine bronze is soldered over the overflow hole to retain food particles.

It may be desirable to construct the samplers of transparent plastic. Through the use of plastic, food particles lodged in the tube during the flushing operation could be detected. It might also be desirable to use steel tubing in the smallest samplers, because rigid thin-walled tubes with a maximum inside diameter for a given outside diameter are desirable.

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STUDY OF A STOCKING OF THE REDEAR AND BLUEGILL IN SOUTHERN ILLINOIS

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IN RECENT YEARS THE REDEAR SUNFISH (Lepomis microlophus) has received a great deal of attention as a fish to be stocked in warm-water ponds and lakes in combination with the largemouth bass (Micropterus salmoides). In some areas it has challenged the stand of the bluegill (Lepomis macrochirus) as a fish to be used for this purpose. Recently a number of experimental stockings were made in southern Illinois. One of these was a combination including both the bluegill and the redear sunfish. The situations resulting from this stocking offered an opportunity to study the relative success of the two species. The stocking was made in Lake Murphysboro, an artificial impoundment located in Jackson County, Illinois.

Lake Murphysboro has a maximum depth of 30 feet and covers an area of 160 acres. The lake's water supply is from surface runoff and a small stream.

The lake filled in 1950, at which time it was stocked with adult largemouth bass, bluegill, redear sunfish, and channel catfish. The present fish population includes the above species plus white crappie (Pomoxis annularis), green sunfish (Lepomis cynellus), warmouth (Chaenobryttus coronarius), yellow bullhead (Ameiurus natalis), and a few miscellaneous forms.

During the summer of 1955, a creel census of the catch by boat fishermen on Lake Murphysboro was conducted. A total of 1,117 man-hours of fishing with worms and cockroaches was recorded. In this amount of fishing, 1,444 bluegill and 295 redear sunfish were caught, providing a rate of catch of 1.29 and 0.26 fish per hour, respectively. During the summer of 1956, a similar creel census was conducted. A total of 3,351 man-hours of fishing with worms and cockroaches was recorded. The catch was 2,600 bluegill and 310 redear; or, in terms of rate, the catch was 0.78 bluegill per hour and 0.09 redear per hour.

In another study, which made use of a trapping device, a count was made of the number of fishes escaping over 33 percent of the spillway of Lake Murphysboro. The following redear were taken: 99 that were from 1 to 5 inches in length and 511 that were from 6 to 10 inches in length. The number of bluegill taken in the same period was as follows: 375 that were from 1 to 5 inches in length and 69 that were from 6 to 9 inches in length. The proportion of each species escaping in each year was approximately the same. The greater loss of redear over the spillway might be interpreted as meaning that the redear population was more dense than the bluegill. Further, observations on loss of fish over the spillway indicated that a greater percentage of the redear population was made up of larger, catchable fish than appeared in the bluegill population. Thus the redear were primarily 6- to 10-inch fish, whereas the bluegill were primarily 1- to 5-inch fish.

To compare the rate of growth and age-class composition of the two species, the scale method was employed. This

COMPARISON OF GROWTH OF THE BLUEGILL AND REDEAR SUNFISH IN LAKE MURPHYSBORO

Year[1/]	Number of specimens	Calculated total length (inches) at annulus: I	II	III	IV	V	VI	VII	VIII	IX
					Redear sunfish					
1955	56	1.8	3.5	5.1	6.4	7.4	7.8	10.2	----	----
1956	160	1.8	3.8	5.3	6.5	7.4	8.8	----	----	----
					Bluegill					
1955	92	1.7	3.0	3.6	5.2	5.9	6.6	7.6	9.3	9.3
1956	120	1.6	2.8	4.0	5.0	5.7	5.9	----	----	----

1/ 1955 specimens were from creel census, whereas 1956 specimens were taken by electrical shocker and traps.

analysis indicated that the redear was a faster growing fish than the bluegill. The difference in growth appears to be of enough magnitude to be of importance in management. The age-class composition of the two fishes appeared to be approximately the same. A spread of ages was represented in both.

A coefficient-of-condition study indicated that the redear was more plump than the bluegill.

Thus, in summary, the only weak point in connection with the redear might be its catchability, but since the redear and the bluegill seem to be compatible, it would appear to be quite sound to stock the two fishes together in combination with the largemouth bass.

Acknowledgment

This study was financed by the Illinois Department of Conservation. Mr. Sam Parr cooperated on behalf of the Department of Conservation.

EFFECT OF SUBSURFACE PERCOLATION ON WATER TREATED WITH ROTENONE

In the fall of 1956, W. L. Minckley, student at Kansas State College, observed the effects of subsurface percolation on wettable-powder rotenone used on a small, intermittent tributary of the Kansas River.

Egg-size gravel, rubble, and some silt formed the stream bottom. Seven holes were dug at 5-foot intervals in the dry stream bed between two pools--the upper pool measuring 60 by 15 feet and 1 to 1.5 feet deep; the lower pool, 40 by 20 feet and 7 feet deep. These holes filled at once, the water passing through with a slight current.

About 20 redbelly dace (Chrosomus erythrogaster) and orangethroat darter (Etheostoma spectabile) were put in each hole and allowed to acclimatize for 1 day. The following day a slurry of 5 percent wettable-powder rotenone was applied by hand to the upstream pool at the rate of 20 pounds per acre-foot. Water temperature ranged from 61° to 64° F., pH was 8.2, and turbidity was 120 p.p.m. Within 15 minutes, both species were showing signs of distress.

The lower artificial holes and pool were checked periodically throughout that day and the next 3 days. No dead fish were seen, and the restrained fish showed no signs of distress. Subsequent seinings in the upstream pool, however, indicated a complete kill in that area.

USE OF STAKE-TYPE BOAT ANCHORS IN WING-NET FISHING

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ANYONE WHO HAS FISHED WING NETS FOR ANY GREAT LENGTH OF TIME has probably seen the need for a method that would allow the nets to be lifted and reset by one man instead of the usual two. Sometime ago I worked out a method which not only is good for one-man use under various wind conditions but also permits a distinct saving of labor for the two-man crew when winds are strong.

In the traditional net-lifting operation, the drift of the boat during the steps of lifting and emptying causes the pot of the net to be pulled out of alignment with the wings. To reset the net with pot and wings in the proper alignment, it is customary for one man to pull on the oars while the other pulls on the tail pole (which is tied to the pot by a long cord). Often net-lifting operations are suspended in windy weather because of the labor involved in pulling the nets back into position for resetting.

The essential feature of the new method is that the successive steps of lifting, emptying, and resetting the net are all carried on from a boat that is anchored in one fixed position. The anchors are two long metal stakes which pass through open-end sockets mounted vertically at each end of the fishing boat (figures 1a and 1b). As the stake-type anchors effectively prevent drift of the boat while the net is being emptied, the usual difficulties of realigning and resetting the net are avoided.

In order that the net operator may be able to reach the tail pole as well as the net hoops from the anchored boat, it will generally be found necessary to reduce the length of the tail cord. The tail cord on the net in figure 1b was shortened from its original length of about 10 feet to about 3 feet.

The stake-type anchors described here have, until now, been used in lake netting

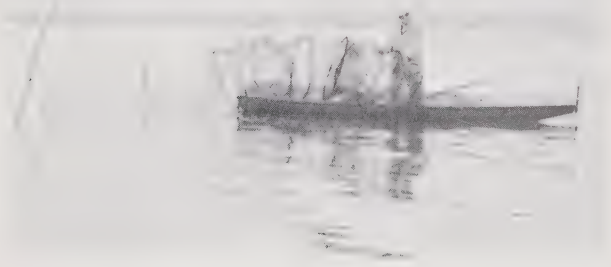

FIGURE 1a.--Lifting a net from an anchored boat, which will stay in this position until the net is reset.

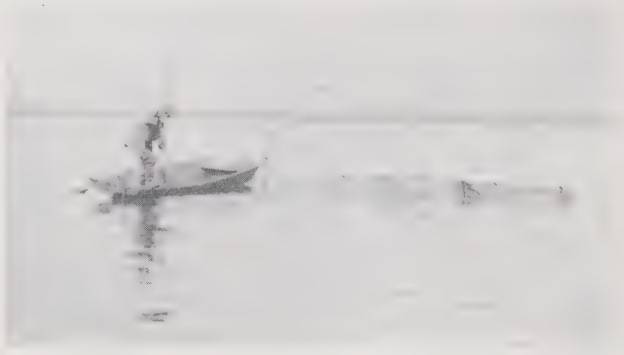

FIGURE 1b.--Use of anchors to hold the boat while putting out net, setting wing poles.

(Illustrations courtesy of the author)

operations at depths of 3 to 4 feet. It appears likely, however, that this same equipment would work satisfactorily at depths of at least 5 to 6 feet. Also, these anchors might be found useful in still-fishing with pole and line around spawning beds and brush piles and in scientific collecting with plankton nets or bottom samplers.

The vertically mounted sockets used for anchoring a 16-foot flat-bottomed boat were made of 1-1/2-inch (inside diameter) gas pipe cut to lengths of 16 inches for the bow (figure 2a) and 12 inches for the stern (figure 2b). The bracket for the bow socket was made of 4-inch channel iron; the bracket for the stern, of 3/16- by 1-3/16-inch strap iron. The sockets were welded to the brackets, and the brackets were mounted to the oak end boards of the boat with 1/2- by 1-1/2-inch lag screws.

In tests it became evident that a stake had to be strong enough to resist permanent bends and yet had to have a certain amount of flexibility. If the boat was anchored with a stake that was too stiff, anyone walking in the boat was thrown off balance by the sudden cessation of the boat's movement. Materials that were too stiff included 3/4-inch gas pipe and 18-gauge soft-drawn steel tubing of 1-1/8-inch outside diameter. A material that was too easily bent was 18-gauge soft-drawn steel tubing of 7/8-inch outside diameter.

The stake that was considered most suitable for our boat was 17-gauge hard-drawn steel tubing of 3/4-inch outside diameter. This was used in 9-foot lengths. Braised to the tubing about 1 foot from one end was a piece of 1/4- by 4-inch steel rod which had been bent to form a hook to support the stake on the top rim of the socket when the stake was not in use. The ends of the stake were closed to keep out water and mud.

Steel tubing of the kind described may be difficult to find in some localities, but any machine-shop should have catalogs of steel-supply companies from which the tubing could be ordered.

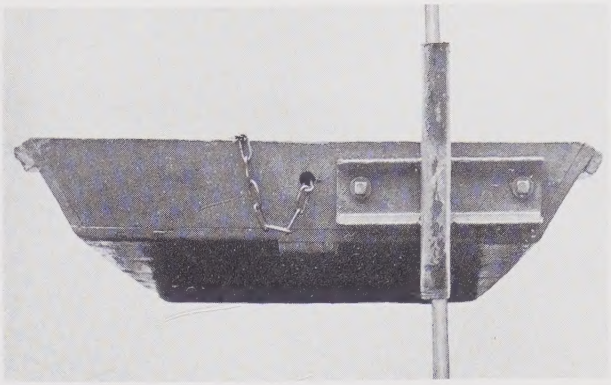

FIGURE 2a.--Mounting bracket for a narrow bow board.

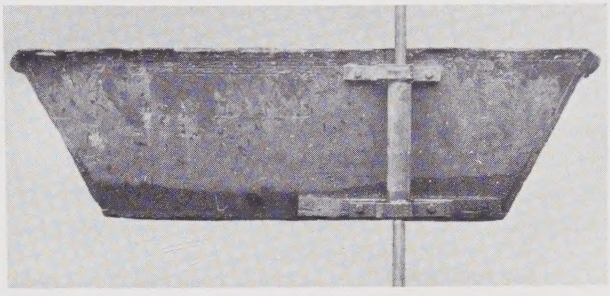

FIGURE 2b.--Mounting bracket for a broad stern board.

As part of the program to control the sea lamprey (Petromyzon marinus) in the Great Lakes, studies to determine the toxicity of 4,346 chemicals to lamprey larvae, rainbow trout (Salmo gairdneri), and bluegill (Lepomis macrochirus) were completed. A rather marked specificity apparently was displayed by some of these compounds. Of the substances to which all three species were exposed, 264 were toxic to only one species (at the levels of concentration and temperature used in these tests), and 333 others were toxic to only two species.

A FIELD TECHNIQUE FOR OBTAINING SAMPLES OF FISH BLOOD SERUM

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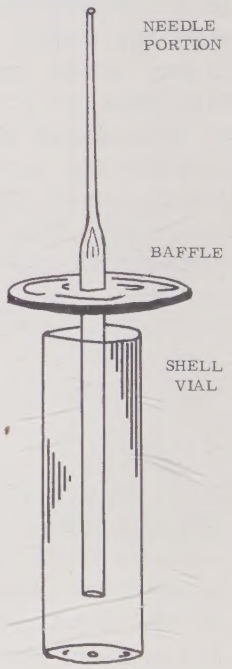
IN CONNECTION WITH RACIAL AND IMMUNOLOGICAL STUDIES of the blood serum of sea herring--studies requiring both pooled and individual samples--it was found that the use of hypodermic needles and syringes was slow and impractical under the field conditions encountered and where the need was for a large number of samples in a short time. It was necessary to use a separate needle for each specimen worked on, because rapid clotting of the blood tended to clog the instrument. Cutting off the tail or slitting the gill arch caused excessive contamination by mucus and other fluids. In view of these difficulties, there has been evolved an alternate technique for the extraction of blood from living fish--a technique that may be of use in other investigations. It is practical for use under any field conditions--from work on the docks to work in the open, pitching dory with a seasick technician. It is rapid and inexpensive and permits the taking of many individual samples with a minimum of equipment.

This technique involves the use of Pasteur pipettes drawn from No. 7 soft glass tubing (outside diameter, 7 millimeters) with the finished inside diameter of the tip dependent on the size of the fish used, as follows:

Standard length of herring (mm.)	Inside diameter of needle end (mm.)
150 to 200	1.5
200 to 250	3.0
250 to 300+	4.0

The pericardium and sinus venosus are pierced from beneath the operculum with a posterior and ventral orientation of the needle. A baffle surrounds the upper middle portion of the needle and prevents contamination by mucus or sea water (see diagram). The baffle (diameter, 2 inches) is made from an ordinary tire tube. A hole made with a leather punch and slightly smaller than the needle lets the baffle fit snugly. The blood is drained by heart action and collected in 8-dram (25- by 95-millimeter) shell vials. After the blood is collected, the vials are put into wooden holding blocks (2- by 3- by 15-inch) with holes bored slightly larger than the vial diameter. The vials are numbered with a glass-marking pencil to correspond with tissue samples and gross observations that are taken simultaneously. The vials are corked at once after blood is taken. The clot is allowed to express, and the serum is removed with pipettes prepared in a manner similar to that for the needles--in fact, they may be interchanged. Thus, needles and pipettes are inexpensive enough to discard if necessary after use under field conditions.



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